VOL. 2 FASC. 3

PSYCHOPHARMACOLOGIA

SPRINGER-VERLAG / BERLIN · GÖTTINGEN · HEIDELBERG

(Postverlagsort Berlin · 24. 4. 1961)

Die Forschung über die Wirkungen und Wirkungsweisen psychotroper Substanzen hat in den letzten Jahren einen unerhörten Aufschwung genommen. Was vordem nur ein erwünschtes Ziel war, ist zu einer neuen Wissenschaft geworden: Psychopharmakologie. Da eine fruchtbare Analyse und Synthese ihrer Probleme nur durch Zusammenarbeit aller Grundfächer (Pharmakologie, Neurochemie, Neurophysiologie, Neurologie, Psychologie und Psychiatrie) möglich wird, ist die Psychopharmakologie eine verbindende, integrierende Forschungsdisziplin. Die ständig anwachsende Literatur dieses komplexen Arbeitsgebietes ist jedoch bisher zwangsläufig über zahlreiche Zeitschriften verstreut, da es bis heute kein Spezialorgan gab, das sich ausschließlich der Psychopharmakologie widmet. Diesem dringenden Bedürfnis zu begegnen, hat sich eine Gruppe von Vertretern der verschiedenen Arbeitsrichtungen der Psychopharmakologie entschlossen, eine neue Zeitschrift „Psychopharmacologia" zu gründen. In ihr sollen die bedeutenden Fortschritte dieses Arbeitsgebietes durch Veröffentlichung experimenteller und klinischer Originalarbeiten, Übersichten der neuesten Literatur sowie kurzer Originalmitteilungen zusammengefaßt werden.

Recent years have witnessed an unprecedented advance in research on the action and effects of psychotropic drugs, and what, formerly, was just a distant goal, has now evolved into a new branch of science: psychopharmacology. As, however, any fruitful analysis and synthesis of its problems can only be attained with the aid of the complete scale of basic sciences (pharmacology, neurochemistry, neurophysiology, neurology, psychology and psychiatry), psychopharmacology constitutes an integrating discipline of research. Owing to the lack of an organ devoted especially to psychopharmacology, the constantly increasing literature pertaining to this complex field of activity has hitherto of necessity been scattered among various periodicals. In order to overcome this drawback, a group of representatives of the various psychopharmacologic sections have engaged in editing a journal, "Psychopharmacologia", in which the publication of original experimental and clinical papers, reviews of recent literature and short original notices will provide a comprehensive survey of the important progress which is being actually achieved in this field of science.

Ces dernières années ont vu un développement sans précédent dans la recherche des effets et du mode d'action des substances psychotropes sur le «Comportement» et ont fait naître une nouvelle science: la Psychopharmacologie. Comme ces problèmes ne peuvent être résolus que par la collaboration des disciplines de base telles que la pharmacologie, la neurochimie, la neurophysiologie, la psychologie et la psychiatrie, la psychopharmacologie est devenue un champ de recherche de première importance. Cependant la littérature toujours croissante en ce domaine de recherche est forcément disséminée dans de nombreux périodiques, puisqu'il n'existe pas encore de journal exclusivement consacré à la psychopharmacologie. Pour répondre à ce pressant besoin un groupe de représentants des diverses disciplines de la psychopharmacologie s'est mis en devoir de rédiger un nouveau journal dans lequel seraient rassemblés les progrès importants de ce domaine, par la publication d'ouvrages originaux expérimentaux et cliniques, ainsi que des rapports sur des questions actuelles.

Richtlinien für die Mitarbeiter siehe am Schluß des Heftes. — Directions to Authors are given at the end of this number. — Directives destinées aux auteurs, voir à la fin du fascicule.

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Psychopharmacologia

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Psychopharmacologia 2, 147—159 (1961)

Original Investigations · Originalarbeiten · Travaux originaux

From the National Institute of Mental Health Addiction Research Center, U. S. Public Health Service Hospital, Lexington, Kentucky

Cross Tolerance Between LSD and Psilocybin

By

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(Received December 10, 1960)

Recently it has been shown (HOFMAN *et al.*, 1958a; DELAY *et al.*, 1958; ISBELL, 1959) that O-Phosphoryl-4-hydroxy-N-dimethyl tryptamine (hereafter referred to as psilocybin), a compound isolated (HOFMAN *et al.*, 1958b) from certain species of mushrooms that are used ceremonially by Mexican Indians (WASSON and WASSON, 1957), has psychotomimetic properties similar to those of the diethylamide of lysergic acid (LSD-25). The close resemblance of the patterns of symptoms induced by LSD and psilocybin suggested that these drugs produce mental aberrations by some common action or by affecting different mechanisms sharing a common final pathway. Since the effects of LSD diminish rapidly when the drug is given daily (ISBELL *et al.*, 1956), it was felt that if the LSD and psilocybin syndromes have a common mechanism, this hypothesis could be further tested by determining if "cross tolerance" between the two drugs existed. In other words, if the degree of the reaction induced by a given dose of psilocybin was significantly less in a person tolerant to LSD, cross tolerance would be said to exist; and, conversely, the reaction to a given dose of LSD should be reduced in a person tolerant to psilocybin. In the latter case it is implied that "direct" tolerance to psilocybin can be developed.

Methods

Experiments. Two experiments were performed at different times. Experiment II was carried out to determine if administration of a larger dose of psilocybin given over a longer period of time than in Experiment I would create a greater degree of tolerance and cross tolerance.

A "cross-over" design using each patient as his own control was employed in both experiments and is summarized in Table 1.

Both experiments consisted of seven periods: (1) *first control*, in which measurements were obtained after the test doses of psilocybin and LSD, (2) *first chronic administration*, in which patients received either psilocybin or LSD once daily in doses increasing to the test level over a period of 6—12 days, (3) *first test of tolerance and cross tolerance*, in which patients were "challenged" with the drug they had been taking

Table 1. *Summary of experimental designs for Experiments I and II*

Period	Expt.	No. of Days	Drugs and Doses		Remarks
			Subjects x[1]	Subjects y[2]	
1. First control	I	7—8	LSD[3] 1.5; Pl[3], Ps 150	Ps[3] 150; Pl, LSD 1.5	To obtain basal data
	II	8—9	LSD 1.5, Pl, Ps 210	Ps 210, Pl, LSD	Order of tests randomized. At least 5 days between Ps and LSD
2. First chronic administration	I	6—7	LSD increasing to 1.5	Ps increasing to 150	To develop tolerance
	II	12	LSD increasing to 1.5	Ps increasing to 210	
3. First test of tolerance and cross-tolerance	I	2	LSD 1.5, Ps 150	Ps 150, LSD 1.5	Test of tolerance and cross tolerance
	II	2	LSD 1.5, Ps 210	Ps 210, LSD 1.5	
4. Withdrawal period	I	7—10	Pl	Pl	To lose tolerance
	II	13	None	None	
5. Second control	I	7—8	Pl, Ps 150, LSD 1.5	LSD 1.5, Pl, Ps 150	To replicate control data
	II	8—9	Pl, Ps 210, LSD 1.5	LSD 1.5, Pl, Ps 210	To test loss of tolerance
6. Second chronic administration	I	6—7	Ps increasing to 150	LSD increasing to 1.5	"Cross-over" to develop tolerance
	II	12	Ps increasing to 210	LSD increasing to 1.5	
7. Second test of tolerance and cross-tolerance	I	2	Ps 150, LSD 1.5	LSD 1.5, Ps 150	Test of tolerance and cross tolerance
	II	2	Ps 210, LSD 1.5	LSD 1.5, Ps 210	

[1] Subjects "x" received LSD chronically, first.

[2] Subjects "y" received psilocybin chronically, first.

[3] LSD = diethylamide of lysergic acid; Pl = placebo; Ps = psilocybin. The order of administration of the drug in each period is indicated by the order in which they appear in the section of the table for that period. Figures after the symbols for the drugs indicate the dose in mcg/kg.

(test of "direct tolerance") and on the subsequent day with the drug they had not been taking (test of "cross" tolerance), (4) *a withdrawal or "washout" period*, in which the patients received placebos (Experiment I) or no drug (Experiment II) in order to lose tolerance, (5) *a second control period*, in which the test doses of LSD and psilocybin were repeated, in order to replicate the control data obtained in the first control period and to determine if tolerance had been completely lost, (6) *second chronic administration*, in which the patients received daily doses of the alternate drug that they had not taken in the first period of chronic administration ("cross-over"), and (7) finally, the *second challenge*, with test doses of LSD and psilocybin as in period 3.

Drugs and Doses. LSD and psilocybin[1] were given in 30 cc of cherry syrup at 8 a.m. with the patients fasting. The syrup, which was used to mask the bitter taste of the psilocybin, served as the placebo. In the first and second control periods the patients received in randomized order 1.5 mcg/kg of LSD, placebo, and 150 mcg/kg (Experiment I) or 210 mcg/kg of psilocybin (Experiment II) before chronic administration of the drugs was begun. Detailed observations were made on these test days. These control experiments were conducted at intervals of at least five days in order that any tolerance conferred by the first drug would be lost.

During the first and second periods of chronic administration the patients in Experiment I received 0.25 mcg/kg of LSD or 25 mcg/kg of psilocybin on the first day. These doses were increased 0.25 mcg/kg (LSD) or 25 mcg/kg (psilocybin) daily until the patients were receiving 1.5 mcg/kg of LSD or 150 mcg/kg of psilocybin on the sixth day. These doses were maintained until the tests of tolerance and cross tolerance were performed. In Experiment II the patients received 0.15 mcg/kg of LSD or 21 mcg/kg of psilocybin on the first day of chronic administration, increasing by 0.15 mcg/kg of LSD or 21 mcg/kg of psilocybin daily until the patients were receiving 1.5 mcg/kg of LSD or 210 mcg/kg of psilocybin on the tenth day. These doses were maintained through the twelfth day. The order in which the patients received the drugs in the first and second periods of chronic administration was randomized in both Experiments I and II. During these periods of chronic administration, detailed observations were not made.

On the first day after completion of the period of chronic administration the patients were "challenged" with the dose of drug they had been receiving (test of direct tolerance). On the second day, they were

[1] We are indebted to Drs. R. Bircher and C. Henze of Sandoz Pharmaceuticals, Hanover, New Jersey, for supplies of psilocybin and diethylamide of lysergic acid tartrate (LSD-25).

challenged with the test dose of the alternate drug (test of cross tolerance). On both of these days detailed measurements were made.

The patients then received placebos for 7—10 days (Experiment I) or no drug for 13 days (Experiment II). It was presumed that the patients would lose any tolerance they had developed, since in previous experiments (ISBELL *et al.*, 1956) tolerance was largely dissipated within three days after discontinuation of LSD.

Following this withdrawal period, second control measurements were obtained after the patients had received in randomized order placebo (Experiments I and II), 1.5 mcg/kg of LSD (Experiments I and II) and 150 mcg/kg (Experiment I) or 210 mcg/kg of psilocybin (Experiment II), with at least five days intervening between adminstration of LSD and psilocybin.

The patients then again received the drugs chronically, those patients who had taken LSD in the first period of chronic administration were given psilocybin according to the schedules described above and vice versa. They were then "challenged" with LSD and psilocybin in the manner described above.

Preliminary Assay. *Experiment II.* Since the test dose of psilocybin (150 mcg/kg) had a lesser degree of effect than the test dose of LSD (1.5 mcg/kg), a preliminary assay was carried out prior to Experiment II. The dose-response curves obtained by ISBELL (1959) were extended and 210 mcg/kg of psilocybin were estimated to be equal to 1.5 mcg/kg of LSD. Accordingly, the above doses of LSD and psilocybin were administered on two occasions at intervals of seven days in random order to 10 subjects. Statistical analyses (see below for method) revealed no significant differences in any of the comparisons made (Table 5, Assay Study).

Subjects. The subjects who volunteered for both experiments were former opiate addicts who were serving sentences for violation of the United States narcotic laws. Their ages varied between 25 to 35 years, all were physically healthy males, and none presented any evidence of the major psychoses. All had psychiatric diagnoses of character or personality disorders, and all had received LSD in previous experiments. Ten subjects served in Experiment I, and 9 in Experiment II.

General Conditions. Subjects were housed in a special ward devoted to clinical research. Temperature, respiratory rate and blood pressure were measured three times daily after the patients had rested quietly in bed during days on which special measurements were not being made. The patients were observed by specially trained aides with long experience in detecting drug-induced changes in behavior.

Observations. During each day of the control periods and the periods of chronic drug administration during which the patients were "chal-

lenged" with placebo, LSD or psilocybin, the following observations were made at hourly intervals, after 10 minutes rest in bed, twice before and eight times after administration of drugs: rectal temperature, pulse rate, systolic blood pressure, pupillary size and threshold for elicitation of the knee jerk. The methods used were those previously described (ISBELL *et al.*, 1956; ISBELL *et al.*, 1959; ISBELL, 1959). In addition, the patients (with the help of an aide) completed a special questionnaire at hourly intervals from 7:30 a.m. to 3:30 p.m. At these same times, general notes on behavior were written. Clinical grades of the intensity of the reaction were assigned on the basis of the system of ISBELL *et al.* (1956).

Analysis of Data. The changes in rectal temperature, pulse and respiratory rates, pupillary size, blood pressure, and threshold for elicitation of the knee jerk after administration of placebo and drugs were calculated by subtracting the average of the two pre-drug observations from the values obtained at various hours. The areas under the time-action curves for each particular measurement composed of these figures were calculated by the method of WINTER and FLATAKER (1950), thus converting all the data on a particular subject, a particular drug, a particular measurement, and a particular day to one figure termed "degree-hours" (temperature), "rate-hours" (pulse rate), etc. The total number of positive responses on the questionnaire were counted over the entire period, eliminating answers which were also scored positively before the drug had been given. Means and standard errors of the means were calculated according to standard statistical techniques.

The difference in the various measurements after placebo, 1.5 mcg/kg of LSD, and 150 or 210 mcg/kg of psilocybin (each individual drug against itself) in the first and second controls were evaluated by a *t*-test for paired observations (EDWARDS, 1946). In Experiment I the only statistically significant difference found between the two sets of controls was a decrease in the pyretogenic effect of psilocybin (Table 2). In Experiment II, significant decreases in the number of positive responses on the questionnaire occurred in the second control (Table 3) after both LSD and psilocybin. Because of these differences in the two controls, the changes in response to the test doses of psilocybin and LSD after chronic administration of either drug were evaluated by comparing the effects of LSD and psilocybin after the first and second periods of chronic drug administration with the corresponding first or second control. In addition, calculations were made using the averages of the two controls. The latter procedure did not alter the significance of the differences greatly, so only the tables showing the differences calculated with the individual first and second controls are presented herein.

Table 2. *Differences in responses to placebo, LSD-25, and psilocybin on first and second controls in Experiment I*

Measure	Placebo	LSD-25	Psilocybin
Temperature	+0.98 ± 0.63	+0.42 ± 0.64	−1.24 ± 0.53*
Pulse rate	−11.53 ± 13.30	−12.62 ± 18.40	−19.60 ± 9.30
Blood pressure	−1.10 ± 15.70	−1.35 ± 12.70	−25.40 ± 11.60
Pupillary size	−0.29 ± 1.65	+0.52 ± 1.18	+0.10 ± 0.83
Knee jerk	−12.56 ± 11.90	−14.83 ± 21.75	+17.88 ± 18.70
Responses on questionnaire	+0.90 ± 1.31	+0.60 ± 6.10	+4.90 ± 9.65
Clinical grade	+0.10 ± 0.10	−0.30 ± 0.20	+0.20 ± 0.41

Figures represent the mean differences ± standard errors of the differences between responses to the same dose of the same drug (placebo, 1.5 mcg/kg of LSD-25 and 150 mcg/kg of psilocybin) on the first and second controls on 10 subjects. None of the differences *except* that for temperature change after psilocybin were significant.

\+ Indicates that the average measurement was increased on the second control.

— Indicates that it was decreased.

* = $P < .05$.

Table 3. *Differences in responses to placebo, LSD-25, and psilocybin in first and second controls in Experiment II*

Measure	Placebo	LSD-25	Psilocybin
Temperature	0.07 ± 0.74	−1.31 ± 0.58	−1.36 ± 0.68
Pulse rate	−9.72 ± 9.81	−37.61 ± 17.69	+1.50 ± 19.50
Blood pressure	+21.44 ± 16.48	−15.66 ± 18.13	6.33 ± 18.98
Pupillary change	−0.10 ± 1.66	+2.00 ± 1.82	+0.43 ± 1.59
Knee jerk	+14.44 ± 6.65	+8.75 ± 23.24	−29.16 ± 19.91
Responses to questionnaire	0	−32.00 ± 12.35*	−29.00 ± 9.29**
Clinical grade	0	−0.55 ± 0.28	−0.38 ± 0.30

Figures represent the mean differences ± the standard errors of the differences between responses to the same doses of the same drug (placebo, 1.5 mcg/kg of LSD, and 210 mcg/kg of psilocybin) in the first and second controls on 9 subjects.

\+ Indicates an increased response on second control.

— Indicates a decreased response on second control.

* Indicates significance < 0.05.

** Indicates significance < 0.02.

The differences in the effects of the two individual drugs (LSD vs psilocybin) were also calculated for both control periods using the same statistical technique for paired observations (Tables 4 and 5).

As explained above, the differences in the response after chronic administration of both LSD and psilocybin were calculated by comparing the responses after first and second chronic administrations of LSD and/or psilocybin with their respective first and second controls. Four different comparisons were made: (1) response to LSD after chronic administration of LSD ("direct" tolerance to LSD), (2) response to psilocybin after chronic administration of LSD ("cross" tolerance to psilocybin), (3) response to psilocybin after chronic administration of

Table 4. *Equivalence of dosage of LSD and psilocybin in Experiment I*

Measure	First Control	Second Control
Temperature	−1.14 ± 0.73	+0.52 ± 0.66
Pulse rate	+24.05 ± 15.26	+31.00 ± 18.11
Blood pressure	+22.75 ± 20.39	+46.80 ± 15.43**
Pupillary size	+4.33 ± 1.24***	+4.74 ± 1.24***
Knee jerk	−6.88 ± 29.80	+25.82 ± 17.81
Responses on questionnaire	+41.40 ± 8.85***	+37.10 ± 16.45*
Clinical grade	+0.60 ± 0.16***	+0.10 ± 0.46

Figures represent mean differences ± standard errors of the differences between responses to LSD-25 (1.5 mcg/kg) and responsesto psilocybin (150 mcg/kg) in 10 subjects, on two separate occasions (1st and 2nd controls).

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

\+ Indicates LSD-25 stronger in effect than psilocybin.

— Indicates psilocybin stronger in effect than LSD-25.

Table 5. *Equivalence of dosage of LSD and psilocybin in Experiment II*

Measure	Assay Study (N = 10)	First Control (N = 9)	Second Control (N = 9)
Temperature	−0.66 ± 0.40	−0.34 ± 0.53	−0.29 ± 0.66
Pulse rate	+25.55 ± 11.98	+65.66 ± 11.84**	+26.56 ± 13.47
Blood pressure	+35.50 ± 22.81	+44.44 ± 22.24	+22.44 ± 16.00
Pupillary change	+2.23 ± 1.20	+1.85 ± 1.60	+3.42 ± 1.37*
Knee jerk	+24.63 ± 29.86	−4.72 ± 28.53	+33.19 ± 21.04
Responses to questionnaire	+9.70 ± 10.74	+16.78 ± 10.98	+13.78 ± 8.98
Clinical grade	−0.05 ± 0.22	−0.11 ± 0.20	−0.28 ± 0.18

Figures represent the mean differences ± the standard errors of the differences between responses to single doses of LSD-25 (1.5 mcg/kg) and responses to psilocybin (210 mcg/kg) on three separate occasions.

\+ Indicates LSD-25 produced a greater response.

— Indicates psilocybin produced a greater response.

* Indicates significance < 0.05.

** Indicates significance < 0.01.

psilocybin ("direct" tolerance to psilocybin), and (4) response to LSD after chronic administration of psilocybin ("cross" tolerance to LSD). The signs of the differences were so arranged that a minus (—) sign indicated a decrease in the measurements after chronic administration as compared with control, and a plus (+) sign indicated an increase.

Since psilocybin has a shorter duration of action than LSD, the differences (except clinical grade) were also evaluated, using values obtained at the peak of both LSD and psilocybin reactions rather than using the area (integrated time action curves) as described above. In addition, the differences were evaluated by a non-parametric rank order test for paired observations (WILCOXON, 1949). The significance of the differences by these statistical techniques agreed well with those obtained by the *t*-test on the time-action (area) figures, so only the differences obtained by the area method are reported in this paper.

Table 6. *Tolerance and cross tolerance, Experiment I*

Measure	After LSD chronically		After psilocybin chronically	
	LSD ("Direct" tolerance)	Psilocybin ("Cross" tolerance)	Psilocybin ("Direct" tolerance)	LSD ("Cross" tolerance)
Temperature	−2.21 ± 0.81*	−0.90 ± 0.73	−1.59 ± 0.46***	−0.22 ± 0.74
Pulse rate	−70.15 ± 11.38***	−18.05 ± 15.22	−21.20 ± 14.46	−10.55 ± 9.20
Blood pressure	−82.35 ± 18.95***	−40.50 ± 8.26***	−23.80 ± 11.97	−21.20 ± 11.85
Pupillary size	−11.04 ± 1.62***	−5.45 ± 1.68**	−3.50 ± 1.28*	−5.43 ± 1.58***
Knee jerk	−54.17 ± 24.86	−58.40 ± 17.67***	−62.12 ± 16.24***	−58.77 ± 20.81**
Responses on questionnaire	−62.20 ± 18.70***	−28.30 ± 9.20**	−10.50 ± 6.21	−57.90 ± 19.30**
Clinical grade	−1.70 ± 0.40***	−1.35 ± 0.32***	−0.85 ± 0.28**	−1.35 ± 0.31***

Figures represent the mean differences ± standard errors of the differences between respective control values and the values found upon testing with LSD-25 (1.5 mcg/kg) of psilocybin (150 mcg/kg) after chronic administration of either drug to 10 subjects.
* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; — Indicates a decrease in response after chronic intoxication.

Table 7. *Tolerance and cross tolerance, Experiment II*

Measure	After LSD chronically (12 days)		After psilocybin chronically (12 days)	
	Test with LSD "direct" tolerance to LSD	Challenge with psilocybin "cross" tolerance to psilocybin	Test with psilocybin "direct" tolerance to psilocybin	Challenge with LSD "cross" tolerance to LSD
Temperature	−1.86 ± 0.65*	−1.65 ± 0.39***	−1.27 ± 0.81	−1.03 ± 0.56
Pulse rate	−49.16 ± 14.57***	−47.44 ± 21.39	−22.11 ± 19.79	−61.66 ± 15.69***
Blood pressure	−44.61 ± 18.37*	−5.66 ± 15.67	−29.38 ± 18.62	−57.83 ± 28.11
Pupillary change	−10.11 ± 2.29***	−9.30 ± 1.47***	−6.89 ± 1.45***	−9.41 ± 2.12***
Knee jerk	−40.97 ± 20.50	−12.69 ± 30.61	−2.41 ± 25.04	−0.97 ± 18.64
Responses to questionnaire	−55.44 ± 17.27**	−39.88 ± 11.07***	−47.11 ± 9.85***	−69.66 ± 13.38***
Clinical grade	− 1.44 ± 0.18***	− 1.38 ± 0.31***	− 2.05 ± 0.21***	−1.88 ± 0.20***

Figures represent the mean differences ± standard errors of the differences between respective control values and the values found upon testing with LSD-25 (1.5 mcg/kg) or psilocybin (210 mcg/kg) after chronic administration of either drug to 9 subjects.
+ Indicates increase in response after chronic intoxication; — Indicates a decrease in response after chronic intoxication.
* Indicates significance < 0.05; ** Indicates significance < 0.02; *** Indicates significance < 0.01.

Results

Controls. The differences in the responses to the same doses of the same drug in first and second controls after placebo, LSD and psilocybin are shown in Tables 2 (Experiment I) and 3 (Experiment II). In Experiment I, the only change that was statistically significant ($p < 0.05$) was a decline in elevation of body temperature after the second control dose of psilocybin. In Experiment II, a significant decline occurred in the number of positive responses on the questionnaire following the second control doses of both LSD and psilocybin. This might indicate that some degree of residual tolerance was still present after 13 days.

Equivalence of Dosage. The differences in the responses to the two different active drugs (LSD and psilocybin) are presented in Tables 4 (Experiment I) and 5 (Experiment II). In Experiment I the responses were generally greater, as indicated by the preponderance of positive signs in Table 4, and these differences were statistically significant on three measures in both the first and second controls. Therefore, in Experiment I, the test dose of psilocybin (150 mcg/kg) was weaker than the test dose of LSD (1.5 mcg/kg). In Experiment II, comparisons were made on three occasions — "assay study", first, and second controls. The majority of the signs in Table 5 are positive, indicating that on the average the effects of 1.5 mcg/kg of LSD were somewhat greater than those of 210 mcg/kg of psilocybin. The differences were, however, statistically significant only in the case of the pulse rate in the first control and the pupillary change in the second control. Since the failure to demonstrate statistically significant differences may have been due to the large variability in some of the measures, the effects of 210 mcg/kg of psilocybin in Experiment II may, therefore, still have been weaker than those of 1.5 mcg/kg of LSD.

Tolerance and Cross Tolerance. The differences in the responses to LSD and psilocybin after chronic administration of either drug an their respective first and second controls are shown in Tables 6 (Experiment I) and 7 (Experiment II). In both tables, the second column shows the difference in response to LSD as compared with the corresponding first or second control after chronic administration of LSD, and reflects "direct" tolerance to LSD. The third column shows the difference in response to psilocybin as compared with the appropriate control after chronic administration of LSD, and reflects "cross" tolerance to psilocybin. Similarly, the fourth column presents measures of "direct" tolerance to psilocybin, and the fifth column "cross" tolerance to LSD.

Inspection of the tables shows that results were very similar in the two experiments. All signs are negative in both tables, indicating an average decrease in response on all measures. In the case of "direct" tolerance to LSD (second columns), the differences reached statistical

significance on six of seven measures in both experiments. In the case of "cross" tolerance to psilocybin (third columns) the differences were statistically significant in five of seven measures (Experiment I), and four of seven measures (Experiment II). In the case of "direct" tolerance to psilocybin (fourth columns), statistically significant change occurred in four measures (Experiment I), and in three measures (Experiment II). In the case of "cross" tolerance to LSD (fifth columns), significant degrees of change occurred in four parameters in both experiments. The measures which reflected "direct" tolerance and "cross" tolerance most clearly were the pupillary diameter, responses on questionnaire and the clinical grades.

Discussion

The data show that a considerable degree of "direct" toleranceto LSD was developed in both Experiments I and II, and that patients "directly" tolerant to LSD also had a considerable degree of "cross" tolerance to psilocybin. Although statistically significant decreases did not occur on as many measures, the data indicate that definite "direct" tolerance to psilocybin was developed and that patients tolerant to psilocybin were "cross" tolerant to LSD. However, under the conditions of these experiments, the degrees of direct tolerance to psilocybin and cross tolerance to LSD were less than the degrees of direct tolerance to LSD and cross tolerance to psilocybin. In this connection, the fact that the direction of change was negative (reduction in the degree of response) may be important even though the differences did not reach statistically significant levels in all parameters. Increasing the dosage and length of time during which psilocybin was administered (Experiment II) did not result in the development of any greater degree of direct tolerance to psilocybin and cross tolerance to LSD than occurred with the lower dosage and shorter period of chronic administration in Experiment I.

The finding that "direct" tolerance to psilocybin and cross tolerance to LSD could not be shown on as many measures might be due to one, or a combination of several factors. In Experiment I, the effects of the dose of psilocybin were definitely less than the effects of the dose of LSD employed, and in Experiment II the effects of the dose of psilocybin prescribed were probably weaker than those of the LSD. Thus the stimulus for the development of tolerance during chronic administration of psilocybin may have been weaker than was the case with LSD. The length of action of psilocybin is shorter than that of LSD and, since only one dose of each drug was given daily, the stimulus for the development of tolerance was not present for as long a time during chronic administration of psilocybin, and the time during which tolerance might be declining, due to lack of sustained drug effect, was greater. Tolerance

to different effects of the two drugs might develop at different rates. Such differential rates of tolerance development occur; for example, with morphine (rapid and nearly complete tolerance to the analgesic effects, slower and only partial tolerance to the miotic and respiratory depressant effects). One might also postulate that LSD and psilocybin have somewhat different mechanisms of action or act on different receptors. It is also possible that failure to demonstrate tolerance and cross tolerance reflects nothing more than the high variability in certain of the measures used (temperature, pulse rate, blood pressure, knee jerk and, responses on the questionnaire). The data are not sufficient for a determination of the relative roles of any of these hypothetical factors.

CERLETTI (1958) did not observe direct tolerance to the pyretogenic effect of psilocybin on daily administration to rabbits, but did find that rabbits "directly" tolerant to LSD were also "cross" tolerant to the temperature-elevating action of psilocybin. Thus the results in the rabbit are similar to those observed in man, and do not help in deciding which of the possible explanations given in the preceding paragraph is the most likely.

BALESTRIERI (1960) did not observe direct tolerance to psilocybin or cross tolerance to psilocybin in patients receiving LSD chronically. The details in BALESTRIERI's paper are not sufficient for a proper evaluation, but the number of patients used was small and the doses of psilocybin employed were low.

The development of "cross" tolerance between LSD and psilocybin reinforces the idea derived from the similarity of clinical effects (ISBELL, 1959) that LSD and psilocybin induce psychic disturbances by some common mechanism, or by different mechanisms which act through a common final pathway. The data, of course, shed no light on the possible nature of such a presumed common action. Biochemical, chemical, neurophysiological or psychological mechanisms (or some combination of them) could be involved.

Summary

1. In two experiments, using a cross-over design, the development of "direct" tolerance to LSD and psilocybin was measured after 10 (Experiment I) or 9 (Experiment II) volunteers had taken LSD in doses increasing to 1.5 mcg/kg over the course of 6—7 days (Experiment I) or 13 days (Experiment II). On another occasion, the same patients received psilocybin in doses increasing to 150 mcg/kg over the course of 6—7 days (Experiment I) or 210 mcg/kg over the course of 13 days (Experiment II).

2. The development of "cross" tolerance to psilocybin in patients "directly" tolerant to LSD was measured by "challenging" the patients, after they had received LSD chronically, with 150 mcg/kg (Experiment I) or 210 mcg/kg (Experiment II) of psilocybin. "Cross" tolerance to LSD was evaluated by "challenging" the patients, after they had received psilocybin chronically, with 1.5 mcg/kg of LSD.

3. A high degree of "direct" tolerance to LSD developed in both experiments, as manifested by statistically significant reductions in six of the seven parameters of response. Patients "directly" tolerant to LSD were also "cross" tolerant to psilocybin on five (Experiment I) or four (Experiment II) parameters.

4. Definite "direct" tolerance also developed after chronic administration of psilocybin in both experiments, but statistically significant reductions occurred in fewer parameters of response (four in Experiment I and three in Experiment II) than was the case with LSD. Patients chronically treated with psilocybin were also "cross" tolerant to LSD on four (Experiment I) or three (Experiment II) measurements. The degree of "direct" tolerance to psilocybin was less than the degree of "direct" tolerance to LSD.

5. The development of "cross" tolerance between LSD and psilocybin reinforces the idea that these two drugs cause psychic disturbances by acting on some common mechanism, or on mechanisms acting through a common final pathway.

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Über den Schutz der Gewebsatmung durch antioxydantisch wirksame Psychopharmaka*

Von

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Mit 1 Textabbildung

(Eingegangen am 10. Oktober 1960)

Wie wir früher berichteten (Wilke, Eberhard und Iizuka 1959), besitzen Chlorpromazin und Reserpin neben ihrer Hemmwirkung auf die Atmung von Homogenaten aus menschlicher Hirnrinde noch antioxydantische Eigenschaften. Sie vermögen dadurch Autoxydationsprozesse zu verhindern, die sich an den ungesättigten Bestandteilen der Gehirnsubstanz abspielen können. Diese antioxydantische Wirkung tritt schon bei niedriger Konzentration der Neuroleptica auf, wobei die Gewebsatmung noch weitgehend geschont bleibt. Da die antioxydantische Wirkung von uns als eine therapeutisch günstige Eigenschaft angesehen wird, prüften wir unter erweiterten Gesichtspunkten die in Tabelle 1 u. 2 aufgeführten psychotropen Substanzen.

Methodik

5—7 weiße Mäuse (männlich, Gewicht 20—25 g) wurden in leichter Chloroformnarkose dekapitiert, die Gehirne isoliert und nach Abspülen unter Zusatz von 25—30 cm³ Ringer-Phosphatlösung p_H 7,4 im Glashomogenisator verarbeitet. Das Homogenat wurde mit Ringerlösung auf 40 cm³ aufgefüllt und nach Entnahme einer Probe zur Bestimmung des Anfangs-Peroxydwertes (s. unten) bis zur weiteren Verarbeitung im Kühlschrank aufbewahrt.

Für die Atmungsmessungen nach der „direkten Methode" von Warburg wurden je 3 cm³ Homogenat in Reaktionsgefäße gegeben, die im Hauptraum 6 mg Glucose (in 0,1 cm³ Wasser) und im Einsatz 0,2 cm³ 5%ige Kalilauge zur Bindung der Kohlensäure enthielten. Das zu untersuchende Neurolepticum wurde in variierten Konzentrationen zugesetzt. Zwei Gefäße ohne Neurolepticum dienten zur Bestimmung der unbeeinflußten Atmung bzw. Autoxydation. Ein Gefäß (Thermobarometer) enthielt statt des Homogenats 3 cm³ Ringerlösung.

Die Messungen erfolgten in der üblichen Weise (Umbreit, Burris und Stauffer 1951, Wilke, Eberhard und Schulz 1959) unter Schütteln der Ansätze mit Sauerstoff bei 36°C, Schüttelgeschwindigkeit:

* Die Arbeit wurde unterstützt durch Mittel des Herrn Kultusministers des Landes Nordrhein-Westfalen und der Max-Planck-Gesellschaft.

82/min, Versuchsdauer 6 Std[1]. Die in der ersten Stunde aufgenommenen Kubikzentimeter O_2, bezogen auf 1 g Trockensubstanz des Homogenats (Q_{O_2} nach WARBURG), dienten als Maß für die Gewebsatmung.

Zur Peroxydbestimmung wurden nach Versuchsende aus den Ansätzen je 1,5 cm³ Homogenat in Zentrifugengläser pipettiert und 2 cm³ Trichloressigsäure (20%ig) sowie 4 cm³ 2-Thiobarbitursäure (0,66%ig) zugesetzt, 15 min lang in ein kochendes Wasserbad gestellt und anschließend 15 min bei 4000—5000 Touren zentrifugiert. Die überstehenden Lösungen wurden dekantiert, mit Wasser auf 10 cm³ aufgefüllt und bei 546 mμ gegen Wasser als Vergleichslösung photometriert (WOLFSON, WILBUR und BERNHEIM 1956). Die gemessenen Extinktionen wurden, auf eine Konzentration von 1 g Trockensubstanz in 1 cm³ Meßlösung bezogen, als Maß für die während der Versuchszeit erfolgte Autoxydation genommen (TBS-Werte).

Die Bestimmung der Trockensubstanz erfolgte durch Eindampfen von 3 cm³ Homogenat (2—3 Proben) bei 110°C im Trockenschrank. Vom Gewicht der getrockneten Masse wurde der Anteil der zugesetzten Ringerlösung (Trockengewicht: 28 mg) abgezogen.

Die Ringer-Phosphatlösung p_H 7,4 enthielt im Liter:

7,08 g NaCl; 0,182 g KCl; 0,193 g $CaCl_2 \cdot 6H_2O$;
0,167 g KH_2PO_4; 3,02 g $Na_2HPO_4 \cdot 2H_2O$.

Chemikalien wurden analysenrein von Merck (Darmstadt), 2-Thiobarbitursäure von Th. Schuchardt (München) bezogen. Die Neuroleptica standen als Ampullenpräparate für klinische Zwecke zur Verfügung. Zum Teil wurden vergleichende Untersuchungen mit Reinsubstanzen durchgeführt.

Photometrische Messungen wurden mit dem lichtelektrischen Photometer „Eppendorf" in Glasküvetten von 1 cm Schichtdicke ausgeführt.

Ergebnisse

Der Einfluß verschiedener Neuroleptica auf Atmung und Autoxydation. Die Versuchsergebnisse sind in Tabelle 1 zusammengestellt. Spalte 3 und 4 enthalten die Atmungs-, Spalte 7 und 8 die Autoxydationswerte in Abhängigkeit von der Konzentration der Neuroleptica (Spalte 2). Ein Vergleich der Werte zeigt, daß die antioxydantische Wirkung bei den meisten der untersuchten Präparate stärker ist als die Hemmwirkung auf die Atmung, da die Autoxydation (TBS-Werte) schon von Neuroleptica-Konzentrationen herabgesetzt wird, die die Atmung noch nicht merklich behindern[2].

[1] Der Zeitraum zwischen der Herstellung der Homogenate und der ersten Druckablesung (Versuchsbeginn) betrug höchstens 1 Std.

[2] Unter den geschilderten Bedingungen zeigten die untersuchten Neuroleptica allein praktisch keine Sauerstoffaufnahme und keinen Einfluß auf den Farbtest mit TBS.

Zur besseren Vergleichsmöglichkeit wurden aus den Meßwerten diejenigen Neuroleptica-Konzentrationen ermittelt, die die Atmung bzw. Autoxydation auf die Hälfte ihres Maximalwertes (Ansatz ohne Neurolepticum) herabsetzen. Diese halbmaximal hemmenden Konzentra-

Tabelle 1. *Der Einfluß verschiedener Neuroleptica auf Atmung und Autoxydation von Homogenaten aus Mäusegehirnen*

Substanz, Präparat, Hersteller	Konzentration in mMol/l	Verbrauchte cm³ O_2/g Trockensubstanz in der 1. Versuchsstunde		6. Versuchsstunde		Peroxydgehalte nach 6 Std O_2-Einwirkung (TBS-Werte)	
		1.	2.	1.	2.	1.	2.
10-(N-Dimethylamino-propyl)-Phenothiazin, Promazin	0,0	—	—	—	—	(20)	(25)
	0,0	11,0	10,7	0,4	0,4	595	600
Protactyl, Asche-Hamburg	0,01	8,7	6,9	0,6	0,3	470	420
1. Versuch Nr. 1011	0,02	7,4	6,4	0,6	0,6	460	395
2. Versuch Nr. 1026	0,05	6,9	7,5	1,3	1,7	440	*335*
	0,1	7,6	6,2	2,6	2,1	*300*	*230*
	0,2	6,2	7,3	2,2	1,9	80	95
	0,5	*6,3*	8,0	1,5	1,6	40	60
	1,0	—	*2,6*	—	0,02	40	20
		$C_{h\,Atm} = 0{,}75$				$C_{h\,Autox} = 0{,}1$	
3-Methoxy-10-(2'-Methyl-3'-Dimethylaminopropyl)-Phenothiazin, Levomepromazin	0,0	—	—	—	—	(15)	(35)
	0,0	7,7	9,8	0,5	0,5	475	590
	0,05	5,4	5,7	1,7	1,4	270	320
	0,1	5,1	6,7	2,1	2,4	*230*	*270*
Neurocil, Bayer-Leverkusen	0,2	4,5	6,1	2,0	2,6	130	135
1. Versuch Nr. 1002	0,5	5,0	*5,8*	1,5	1,7	80	60
2. Versuch Nr. 1004	1,0	*4,7*	*4,2*	0,6	0,3	20	40
	2,0	*0,8*	0,06	0,01	0,07	15	25
		$C_{h\,Atm} = 1{,}0$				$C_{h\,Autox} = 0{,}1$	
10-(3'-(4''-Methylpiperazinyl)-Propyl)-Phenothiazin, Perazin	0,0	—	—	—	—	(50)	(45)
	0,0	7,8	11,4	0,3	2,0	750	720
	0,01	6,2	9,2	0,6	2,5	560	550
Taxilan, Promonta-Hamburg	0,02	6,1	—	1,2	—	465	—
1. Versuch Nr. 1014	0,05	5,3	8,0	2,3	5,2	*375*	*300*
2. Versuch Nr. 1015	0,1	6,8	6,3	3,5	4,4	230	195
	0,2	6,3	—	3,9	—	80	—
	0,5	7,6	*5,7*	4,6	2,1	75	50
	1,0	*2,7*	2,9	0,4	0,2	40	30
		$C_{h\,Atm} = 0{,}75$				$C_{h\,Autox} = 0{,}05$	
3-Chlor-10-(N-Dimethylaminopropyl)-Phenothiazin, Chlorpromazin	0,0	—	—	—	—	(25)	(35)
	0,0	7,7	11,4	0,5	0,7	520	790
	0,01	6,3	7,3	0,5	0,3	415	535
Megaphen, Bayer-Leverkusen	0,02	7,3	6,4	0,6	0,8	480	545
	0,05	6,1	6,9	1,0	0,9	410	*435*
1. Versuch Nr. 1003	0,1	—	6,4	—	1,5	—	*320*
2. Versuch Nr. 1018	0,2	—	*6,4*	—	1,7	—	295
	0,5	5,0	*3,6*	0,9	0,1	90	90
	1,0	*3,4*	—	0,3	—	30	35
		$C_{h\,Atm} = 0{,}5$				$C_{h\,Autox} = 0{,}075$	

tionen (C_h) erlauben einen zahlenmäßigen Vergleich der untersuchten Substanz. Für einen günstigen Therapieeffekt sollte nach unserer Ansicht der für die Autoxydation charakteristische C_h-Wert ($C_{h\,\mathrm{Autox}}$) im Vergleich zu dem C_h-Wert für die Atmung ($C_{h\,\mathrm{Atm}}$) möglichst niedrig sein,

Tabelle 1 (Fortsetzung)

Substanz, Präparat, Hersteller	Konzentration in mMol/l	Verbrauchte cm³ O_2/g Trockensubstanz in der 1. Versuchsstunde		6. Versuchsstunde		Peroxydgehalte nach 6 Std O_2-Einwirkung (TBS-Werte)	
		1.	2.	1.	2.	1.	2.
3-Chlor-10-((4'-(2''-Hydroxyäthyl)-piperazinyl-)-Propyl)-Phenothiazin, Clorperphenazin	0,0	—	—	—		(30)	(35)
	0,0	7,5	11,8	0,3	0,6	470	640
	0,01	—	8,6	—	0,4	—	480
	0,02	6,1	8,2	0,3	0,6	—	500
Decentan, Merck-Darmstadt	0,05	5,7	8,7	0,4	0,9	355	480
1. Versuch Nr. 1005	0,1	5,5	9,7	1,4	1,8	*240*	*410*
2. Versuch Nr. 1006	0,2	4,5	7,6	2,2	3,1	145	*260*
	0,5	*3,6*	*6,0*	1,4	3,1	55	110
	1,0	2,0	—	0,6	—	30	—
		$C_{h\,\mathrm{Atm}}=0{,}5$				$C_{h\,\mathrm{Autox}}=0{,}1$	
10-(N-Dimethylaminopropyl)-Thiophenylpyridylamin, Prothipendyl	0,0	—	—	—	—	(65)	(20)
	0,0	14,9	14,5	1,3	0,7	870	835
	0,02	7,3	7,3	0,8	0,5	555	585
Dominal, Chemiewerk-Homburg	0,05	7,0	6,7	0,9	0,4	490	585
	0,1	9,9	5,6	1,5	0,4	595	455
1. Versuch Nr. 1007	0,2	10,3	8,0	1,3	0,7	710	535
2. Versuch Nr. 1008	0,5	10,2	*7,4*	1,4	0,5	635	500
	1,0	*7,9*	*7,1*	0,6	0,5	*490*	*505*
	2,0	—	4,4	—	0,2	—	*360*
		$C_{h\,\mathrm{Atm}}=1{,}0$				$C_{h\,\mathrm{Autox}}=1{,}5$	
1-(N-Dimethylaminopropyl)-4,5-Dihydro-2,3-6,7-Dibenzoazepin	0,0	—	—	—	—	(35)	(15)
	0,0	10,3	7,4	1,1	0,5	620	420
	0,005	8,3	—	1,1	—	475	—
Tofranil, Geigy-Basel	0,01	9,3	4,6	1,8	0,4	475	250
1. Versuch Nr. 1009	0,02	8,2	4,8	1,6	1,0	475	235
2. Versuch Nr. 1025	0,05	8,1	4,5	1,9	1,2	340	*205*
	0,1	8,1	5,2	2,6	1,6	*345*	195
	0,2	7,7	5,3	2,5	1,1	*260*	150
	0,5	*8,1*	*5,0*	1,7	0,8	190	125
	1,0	*0,9*	—	0,03	—	70	—
		$C_{h\,\mathrm{Atm}}=0{,}75$				$C_{h\,\mathrm{Autox}}=0{,}1$	
2-Chlor-9-(N-Dimethylaminopropyliden)-Thioxanthen, Clorprothixen	0,0	—	—	—	—	(25)	(25)
	0,0	10,3	8,5	3,0	0,7	350	500
	0,01	6,9	5,2	2,2	0,4	400	370
	0,02	6,8	4,8	2,4	0,5	395	365
Truxal, Troponwerke-Köln	0,05	5,9	5,0	2,4	0,6	395	370
1. Versuch Nr. 1019	0,1	*5,7*	5,1	2,5	0,8	385	315
2. Versuch Nr. 1027	0,2	*4,6*	—	2,1	0,7	*255*	*290*
	0,5	4,5	*3,9*	0,9	0,5	200	130
	1,0	1,1	—	0,0	—	50	—
	2,0	0,7	—	0,0	—	25	—
		$C_{h\,\mathrm{Atm}}=0{,}2$				$C_{h\,\mathrm{Autox}}=0{,}2$	

Tabelle 1 (Fortsetzung)

Substanz, Präparat, Hersteller	Konzentration in mMol/l	Verbrauchte cm³ O_2/g Trockensubstanz in der 1. Versuchsstunde		6. Versuchsstunde		Peroxydgehalte nach 6 Std O_2-Einwirkung (TBS-Werte)	
		1.	2.	1.	2.	1.	2.
2-Keto-3-Isobutyl-9,10-Dimethoxy-Hexahydrobenzo-(a)-Chinolizin	0,0	—	—	—	—	(30)	(25)
	0,0	9,0	9,5	1,8	0,6	610	550
	0,02	6,8	—	1,8	—	515	—
Nitoman, Hoffman-La Roche, Grenzach (Baden)	0,05	6,1	—	2,2	—	400	—
	0,1	5,8	*5,9*	2,7	2,1	*380*	70
(Versuchspräparat, nicht im Handel)	0,2	*5,6*	*4,3*	4,5	1,7	120	60
	0,5	*3,6*	4,1	4,1	3,2	60	55
1. Versuch Nr. 1020	1,0	2,9	2,4	3,8	2,7	70	50
2. Versuch Nr. 1021	2,0	2,8	2,3	3,1	1,7	60	45
	5,0	—	2,0	—	1,7	—	40
		$C_{h\,Atm}$ = 1,0				$C_{h\,Autox}$ = 0,1	
Reserpin (Alkaloid)	0,0	—	—	—	—	(65)	(35)
Sedaraupin, Boehringer u. S. Mannheim	0,0	12,9	11,4	1,5	2,5	655	700
	0,01	7,6	—	0,9	—	590	—
1. Versuch Nr. 1016	0,02	7,7	9,4	1,2	2,4	500	635
2. Versuch Nr. 1017	0,05	6,0	11,1	1,2	2,5	510	640
	0,1	6,7	7,5	1,7	2,0	495	475
	0,2	5,0	7,8	2,3	3,1	*320*	*415*
	0,5	5,8	6,4	3,2	3,7	65	175
	1,0	*6,4*	*6,2*	1,2	2,0	45	65
	2,0	4,4	5,6	0,6	0,6	75	75
		$C_{h\,Atm}$ = 0,2				$C_{h\,Autox}$ = 0,2	

3 cm³ Homogenat in Ringer-Phosphatlösung p_H 7,4 unter Zusatz von 6 mg Glucose und der angegebenen Menge Neurolepticum. „Direkte Methode" von WARBURG mit reinem Sauerstoff bei 36°C. Schüttelfrequenz: 82/min. Im Einsatz der Gefäße 0,2 cm³ 5%ige KOH.

Peroxydbestimmung mit 2-Thiobarbitursäure (TBS) nach 6 Std O_2-Einwirkung. Die in der Tabelle vorkommenden Abkürzungen entsprechen denen im Text. Die Ziffern 1. und 2. über den Spalten 3—8 verweisen auf die in der 1. Spalte angegebenen Versuchsnummern (je 2 Parallelversuche mit verschiedenen Homogenaten).

Aus den kursiv gedruckten Werten in der 3., 4., 7. und 8. Spalte wurden die C_h-Werte ermittelt. Die in der 7. und 8. Spalte jeweils am Kopf der Kolonnen stehenden TBS-Werte beziehen sich auf den Ausgangszustand des betreffenden Homogenats (Nullwerte).

da dann die antioxydantische Wirkung gegenüber der atmungshemmenden überwiegt. Der Quotient $C_{h\,Atm}$: $C_{h\,Autox}$ sollte also möglichst groß sein. Ordnet man die untersuchten Präparate nach diesem Gesichtspunkt, dann ergibt sich die aus Tabelle 2 zu ersehende Reihenfolge.

Die chlorfreien Phenothiazinderivate (Taxilan, Neurocil und Protactyl) gehören zu den gut antioxydantisch wirksamen Substanzen. Ihre autoxydationshemmende Wirkung ist um eine Größenordnung stärker als ihre Hemmwirkung auf die Atmung. Das schwefelfreie Tofranil bildet den Übergang zur etwas schwächeren Mittelgruppe mit den chlor-

Tabelle 2

Substanz	Präparat	$C_{\frac{1}{2}}$ Atm in mMol/l	$C_{\frac{1}{2}}$ Autox in mMol/l	$\frac{C_{\frac{1}{2}}\text{ Atm}}{C_{\frac{1}{2}}\text{ Autox}}$
10-(3′-(4″-Methylpiperazinyl)-Propyl)-Phenothiazin	Taxilan	0,75	0,05	15
3-Methoxy-10-(2′-Methyl-3′-Dimethylaminopropyl)-Phenothiazin	Neurocil	1,0	0,1	10
10-(N-Dimethylaminopropyl)-Phenothiazin	Protactyl	0,75	0,1	7,5
1-(N-Dimethylaminopropyl-4,5-Dihydro-2,3-6,7-Dibenzoazepin	Tofranil	0,75	0,1	7,5
3-Chlor-10-(N-Dimethylamino-propyl)-Phenothiazin	Megaphen	0,5	0,075	6,7
3-Chlor-10-((4′-(2″-Hydroxy-äthyl)-piperazinyl-)-Propyl)-Phenothiazin	Decentan	0,5	0,1	5
Reserpin	Sedaraupin	1,0	0,2	5
2-Keto-3-Isobutyl-9,10-Dimeth-oxy-Hexahydrobenzo-(a)-Chinolizin	Nitoman	0,2	0,1	2
2-Chlor-9-(N-Dimethylamino-propyliden)-Thioxanthen . .	Truxal	0,2	0,2	1
10-(N-Dimethylaminopropyl)-Thiophenylpyridylamin . . .	Dominal	1,0	1,5	0,7

haltigen Phenothiazinderivaten Megaphen und Decentan, sowie Reserpin. Nitoman leitet zu den schwachen Antioxydantien Truxal und Dominal über, bei denen antioxydantische und atmungshemmende Wirkung praktisch zusammenfallen. Bei der unterschiedlichen chemischen Zusammensetzung der einzelnen Präparate lassen sich engere Zusammenhänge zwischen der chemischen Struktur und der antioxydantischen Wirkung nicht feststellen.

Der Einfluß antioxydantisch wirkender Neuroleptica auf die Alterung von Homogenaten aus Mäusegehirnen. Zur Erzielung möglichst hoher Peroxydwerte in den Homogenaten dehnten wir die Dauer unserer Autoxydationsversuche auf 6 Std aus und verfolgten über diesen längeren Zeitraum auch die Atmung. In allen Versuchen fiel der Sauerstoffverbrauch mit fortschreitender Versuchszeit von einem Höchstwert in der ersten Versuchsstunde bis auf einen Restwert in der letzten Stunde ab. Dieses Absinken der Atmung kann als Folge der fortschreitenden Alterung der Homogenate angesehen werden. Geschwindigkeit und Stärke dieses Alterungsprozesses variieren bei den einzelnen Homogenaten.

Über die Ursache der an sich schon bekannten Alterungsvorgänge selbst wurden unseres Wissens bisher noch keine näheren Untersuchungen angestellt, da sie anscheinend zwanglos aus der Erschöpfung der Atmungssubstrate erklärt werden können. Mann und Quastel (1946)

sowie DICKENS (1946) vermuten als Ursache für das schnellere Absinken der Atmung von Hirngewebe bei höheren Sauerstoffdrucken die Vergiftung von sauerstoffempfindlichen Enzymen des Atmungsstoffwechsels. Aus unseren Versuchen läßt sich aber ableiten, daß auch Autoxydationsprozesse an den Ursachen der Alterung in den Homogenaten beteiligt sind. Wir machten nämlich die Beobachtung, daß der Alterungsvorgang verzögert wird, wenn die Ansätze ein antioxydantisch wirksames Neurolepticum enthalten. Dieser Effekt zeigt sich bei solchen Neuroleptica-Konzentrationen, die die Atmung nur schwach, die Autoxydation aber deutlich herabsetzen. Bezeichnenderweise ist er gerade bei den schwach antioxydantischen Präparaten kaum oder gar nicht festzustellen.

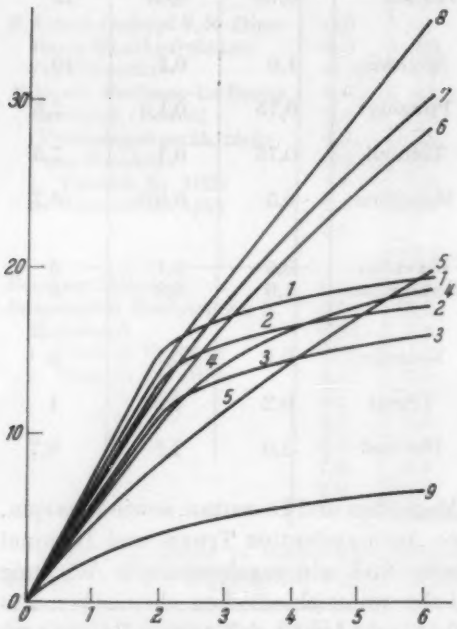

Abb. 1. Wirkung eines antioxydantischen Psycholepticums auf die Atmung eines Homogenats aus Mäusegehirnen. Versuch Nr. 1014. Versuchsbedingungen wie bei Tabelle 1 angegeben. Abszisse: Zeit in Stunden. Ordinate: Aufgenommene cm^3 O_2, bezogen auf 1 g Trockensubstanz. Kurven *1* und *2*: ohne Zusatz. Kurve *3*: Mit 0,01 mMol Taxilan/l. Kurve *4*: Mit 0,02 mMol Taxilan/l. Kurve *5*: Mit 0,05 mMol Taxilan/l. Kurve *6*: Mit 0,1 mMol Taxilan/l. Kurve *7*: Mit 0,2 mMol Taxilan/l. Kurve *8*: Mit 0,5 mMol Taxilan/l. Kurve *9*: Mit 1,0 mMol Taxilan/l

Da die Geschwindigkeit der Alterung ebenso wie Sauerstoffaufnahme und Peroxydbildung von Homogenat zu Homogenat stark variiert, können die mit verschiedenen Homogenaten erhaltenen Meßwerte nicht zu einem quantitativen Vergleich der Präparate dienen. Der Effekt zeigt sich naturgemäß am stärksten bei einem rasch alternden Homogenat; bei einem langsam alternden Homogenat ist er weniger auffällig. Diese im Homogenat liegenden Faktoren überdecken z.T. die Wirkung der untersuchten Präparate.

Ein günstiges Beispiel für den Effekt eines antioxydantisch aktiven Psychopharmakons auf die Alterung der Gewebsatmung ist in Abb. 1 dargestellt. Das verwendete Homogenat zeigte schnelle Alterung (Kurven 1 und 2). Bei Zusatz von Taxilan in steigender Konzentration wird die Alterung zunehmend verzögert, die entsprechenden Kurven (3—8) erscheinen immer weniger gekrümmt. Zu hohe Konzentration hemmt dann auch die Atmung (Kurve 9).

Durch Vergleich des Sauerstoffverbrauchs in der letzten Versuchsstunde läßt sich dieser Effekt innerhalb der gleichen Meßreihe, also am gleichen Homogenat, auch zahlenmäßig auswerten. Dies wurde in der 5. und 6. Spalte von Tabelle 1 durchgeführt. Sie enthalten ähnlich wie die Spalten 3 und 4 die Sauerstoffaufnahme, jedoch in der 6. Versuchsstunde, als Maß für die Alterung, in Abhängigkeit von der Konzentration der untersuchten Neuroleptica.

Der Vergleich läßt erkennen, daß alle von uns untersuchten Neuroleptica im Bereich ihrer antioxydantisch wirksamen Konzentrationen das Absinken der Gewebsatmung mehr oder weniger stark verzögern. Lediglich Truxal und Dominal, die nach unseren Untersuchungen keinen nennenswerten antioxydantischen Effekt besitzen, lassen auch keine Schutzwirkung auf die Atmung erkennen.

Unsere Versuche lassen den Schluß zu, daß die in der Abnahme der Gewebsatmung erkennbare Alterung der Homogenate vorwiegend eine Folge von Autoxydationsprozessen ist, da sie durch geeignete Antioxydantien verzögert wird. Wäre die Alterung der Homogenate lediglich eine Folge des Verbrauchs von Atmungssubstraten, so könnte sie durch Antioxydantien allein nicht aufgehalten werden.

Homogenate, die wir unter sonst gleichen Bedingungen mit Luft anstelle von reinem Sauerstoff behandelten (vgl. Mann und Quastel 1946), zeigten langsamere Alterung und verringerte Peroxydbildung nach 6 Std. Dies deutet ebenfalls darauf hin, daß die Alterung der Homogenate durch Autoxydationsvorgänge verursacht wird.

Beziehung zwischen Atmung und Autoxydation von Hirngewebshomogenaten. Die durch Autoxydationsvorgänge bewirkte Herabsetzung der Gewebsatmung wird aller Wahrscheinlichkeit nach durch die entstehenden Lipoperoxyde verursacht.

So ist z.B. Wasserstoffperoxyd ein starkes Zellgift; seine Anreicherung im Gewebe wird durch die fast überall vorhandene Katalase verhindert. Für andere wasserlösliche Hydroperoxyde gilt das gleiche (Theorell 1948; Kern et al. 1959). Auch in Wasser schwerlösliche Peroxyde, z.B. Dibenzoylperoxyd, schädigen die Zellatmung. Gleichzeitig können sie ihre Eigenschaft als Autoxydationskatalysatoren entfalten, d.h. die Bildung von Lipoperoxyden beschleunigen.

Die pathologische Wirkung der Lipoperoxyde untersuchten Dam 1944, Dam und Granados 1945 sowie Muset 1958, Muset et al. 1959. Nach der Behandlung mit Sauerstoff findet man in Homogenaten aus Hirngewebe, denen Dibenzoylperoxyd (DBP) zugesetzt wurde, mit Thiobarbitursäure (TBS) erhöhte Peroxydwerte[1], verglichen mit den peroxydfreien Ansätzen, bei gleichzeitig verringerter Zellatmung. Ansätze

[1] DBP reagiert selbst nicht mit TBS (Glavind und Hartmann 1951; Wilke, Eberhard und Schulz 1959).

mit und ohne Zusatz von 15 mg DBP zu 3 cm³ Homogenat aus menschlicher Hirnrinde zeigten folgendes Verhalten:

Tabelle 3

	cm³ O_2/g Trockensubstanz		TBS-Wert
	nach 1 Std	nach 8 Std	(Anfangswert) 10
ohne Zusatz . .	2,3	12,6	220
mit 15 mg DPB .	1,2	5,1	320

Im Ansatz mit DBP ist die Atmung herabgesetzt, die Autoxydation dagegen gesteigert gegenüber dem peroxydfreien Ansatz. Das Peroxyd beschleunigt also die Autoxydation, hemmt aber gleichzeitig die Atmung. Weitere Versuche zeigten ein ähnliches Ergebnis.

Im Zusammenhang mit dem früher Gesagten ergibt sich, daß autoxydationsfördernde Substanzen die Atmung hemmen, während antioxydantisch wirksame Substanzen die Atmung zu schützen vermögen.

Diskussion

Die Phenothiazinderivate und chemisch ähnlich strukturierte Verbindungen haben in den letzten Jahren immer mehr an Bedeutung gewonnen. Hinsichtlich ihrer Verträglichkeit sind sie den früher vorwiegend verwendeten Barbituraten überlegen. Die von der praktischen klinischen Erfahrung gesteuerte Entwicklung führte sicher nicht zufällig von Substanzen ohne antioxydantische Eigenschaften zu solchen mit z.T. ausgeprägtem Charakter als Antioxydantien. Unserer Meinung nach stellt die antioxydantische Wirkung eine nicht nur günstige, sondern sogar notwendige Eigenschaft atmungshemmender Neuroleptica dar. In Zusammenhang mit dieser Vorstellung hatte die vorliegende Arbeit das Ziel, in den letzten Jahren in Gebrauch gekommene psychotrope Substanzen auf ihre atmungshemmende und antioxydantische Wirkung an einem der lebenden Hirnsubstanz möglichst ähnlichen Material zu prüfen.

Die Bedeutung von Autoxydationsprozessen im Gehirn wird klar, wenn man sich die chemische Besonderheit des Hirngewebes vor Augen hält. Hinsichtlich seiner Stoffwechselaktivität steht das Gehirn mit an erster Stelle. Seine Trockensubstanz besteht beim Menschen zu 24% aus Phosphatiden, die sich durch einen besonders hohen Gehalt an hochungesättigten Fettsäuren auszeichnen (Klenk 1931, 1932, 1951; Klenk und Bongard 1952; Klenk und Lindlar 1955). Diese neigen besonders leicht zur Autoxydation (Schulz und Wilke 1955, 1956). Der hierfür nötige Sauerstoff ist im Hirngewebe frei gelöst an allen Stellen und zu jedem Zeitpunkt im Überschuß vorhanden (Opitz und

Schneider 1950). Normalerweise wird er für den Atmungsstoffwechsel verbraucht, ohne daß Autoxydationsprozesse in Gang kommen. Bei starker Einwirkung eines antioxydantisch nicht aktiven Sedativums können jedoch im Koma Autoxydationsprozesse starten (Wilke, Eberhard und Iizuka 1959). Ein begünstigender Umstand hierfür ist der extrem niedrige Katalasegehalt des Gehirns (Dickens 1946; Muset 1958). Es erscheint uns daher als ein wichtiger Befund, daß bei den meisten der von uns untersuchten Präparate die antioxydantische Wirkung wesentlich stärker ist als die Hemmwirkung auf die Gewebsatmung. Das bedeutet, daß das Gehirn auch bei längerer Einwirkungsdauer dieser atmungshemmenden Neuroleptica vor Autoxydation geschützt bleibt.

Barbiturate wie Nembutal besitzen keine antioxydantische Wirkung (Wilke, Eberhard und Schulz 1959; Wilke, Eberhard und Iizuka 1959). Die Auswirkung sich im Gehirn vollziehender Autoxydationsprozesse wurde in unseren früheren Arbeiten gezeigt (Wilke, Eberhard und Iizuka 1959; Wilke und Iizuka 1960; Iizuka und Wilke 1960). Die bei den Versuchstieren im Nembutalkoma entwickelte Hirnschwellung wird durch prophylaktische Gaben von Tocopherol verhindert, dessen antioxydantische Wirkung bekannt ist (Dam 1953). Auch das antioxydantisch aktive Megaphen erzeugt bei stärkster Sedierung keine Hirnschwellung. Die Entstehung der Hirnschwellung als Folge von Autoxydationsprozessen kann also durch Antioxydantien verhindert werden.

Elektronenoptisch läßt sich zeigen, daß die Mitochondrien in der Hirnrinde der Versuchstiere unter Nembutal schrumpfen. Bei den prophylaktisch mit Tocopherol behandelten Tieren tritt keine Schrumpfung, sondern eine Entfaltung ein. Das gleiche Bild sieht man bei den mit Megaphen behandelten Tieren (Wilke und Iizuka 1960; Iizuka und Wilke 1960). Auch ultrastrukturell ergibt sich somit der Hinweis, daß Megaphen eine dem Nembutal fehlende Schutzfunktion besitzt, die durch das antioxydantisch wirkende Tocopherol ersetzt werden kann.

Die schon länger bekannte Irreversibilität der Tocopherol-Avitaminosen, deren Folgen schon im Frühzustand nicht mehr durch nachträgliche Vitamingaben rückgängig gemacht werden können (Einarson 1954), läßt sich mit den Verhältnissen bei chemisch-technischen Autoxydationsprozessen vergleichen: Auch hier verhindern Antioxydantien zwar den Start, können aber einen schon angelaufenen Autoxydationsprozeß nicht mehr rückgängig machen. Dam u. Mitarb. (1951) versuchten schon früher im Tierexperiment Tocopherol durch technische Antioxydantien wie Phenothiazin zu ersetzen.

Faßt man unsere bis jetzt vorliegenden Ergebnisse übersichtlich zusammen, so läßt sich über die Zusammenhänge zwischen Atmung und Autoxydation im Gehirn folgendes sagen:

1. Die Hemmung der Atmung durch Barbiturate begünstigt die Autoxydation.

2. Die Hemmung der Autoxydation durch Antioxydantien schont die Atmung.

3. Die Beschleunigung der Autoxydation durch Peroxyde schädigt die Atmung.

Diese in vitro beobachteten Effekte geben einen Hinweis für die Deutung unserer Versuche in vivo (WILKE, EBERHARD und IIZUKA 1959; WILKE und IIZUKA 1960; IIZUKA und WILKE 1960). Sie weisen auf die Funktion eines antioxydantisch wirksamen Prinzips als Schutzfaktor für die Atmung des Hirngewebes hin. Für die Therapie ergibt sich aus dem Gesagten, daß das Gehirn bei therapeutischer Anwendung atmungshemmender Neuroleptica vor Autoxydationsprozessen geschützt werden sollte.

Summary

The effect of clinically frequently applicated psychotropic substances in regard to respiration and autoxidation has been examined on homogenized brains of white mice. According to the antioxidative effect the Psychopharmaca were characterized as good, middle or weak antioxidants. The antioxidative active Neuroleptica in favourable doses show a retarding effect on the aging of tissue respiration of the brain homogenates. From this observation in relation with other experimental results is concluded:

1. The inhibition of respiration favours autoxidation.
2. The inhibition of autoxidation preserves respiration.
3. The acceleration of autoxidation damages respiration.

The importance of antioxidative effect of psychotropic substances is pointed out in regard to the therapy.

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Impairment of Retention for a Conditioned Response by Ether Anesthesia in Mice*

By

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With 2 Figures in the Text

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The effects of anesthesia on conditioned behavior have been shown to be capable of limiting both acquisition (LEUKEL, 1957) and retention (PEARLMAN et al. 1959) to some degree. Since retention appears to rely upon neural activity, one might assume that the changes in neural activity induced by anesthesia would affect the retention of events just prior to the anesthesia. To test the hypothesis that a general anesthetic agent, diethyl ether, impairs the retention of an avoidance response established immediately prior to the anesthesia, a single-trial conditioning situation was used (JARVIK and ESSMAN 1960).

The methodological advantages in the use of such a technique are that: (1) it allows for the use of mice-expendable, inexpensive subjects that are ordinarily quite difficult to work with in conditioning studies; (2) it can be carried out in a relatively short period of time; (3) it may be used with several species; (4) it provides for the establishment of a stable conditioned avoidance response which can be readily measured.

In a situation where responses are used to determine the effects of ether, one must be able to differentiate between the effects of the anesthesia on performance per se as well as on retention. If retention is influenced by events which produce a transient disturbance of excitatory threshold within a half-hour after learning, as SPERRY (1959), among others, has viewed the formation of memories, then any effect of ether introduced one hour following conditioning should not affect the consolidation of the memory trace.

Methods

Subjects. Subjects were 120 male Swiss Webster albino mice, 21 days old, weighing approximately 20 gm.

Apparatus. The apparatus, shown in Fig. 1, consisted of a stationary metal platform projecting from the center of a 24 inch square masonite wall. A sliding metal shelf $^1/_2$ inch below the platform could be pulled

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out. A 400 V, 60 cycle power supply in series with 200,000 ohms was connected to the platform and shelf for the experimental subjects.

Procedure. Subjects were divided into six groups of 20 subjects each and were treated as follows:

Group I (Shocked-Ether). Subjects were individually placed on the platform (*A*), and the shelf (*B*) was pulled out. A voltage difference applied between the platform and shelf allowed for a 2 MA shock to be

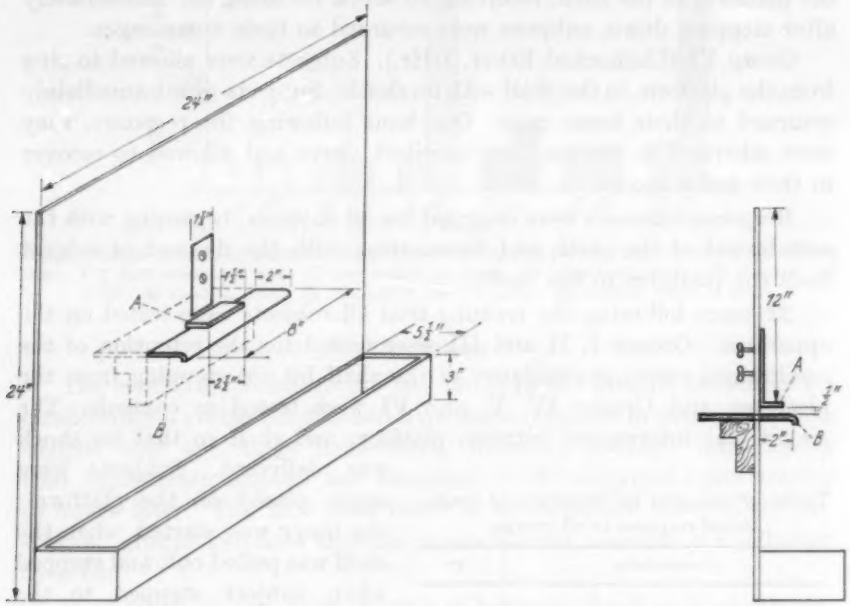

Fig. 1. Schematic view of the apparatus. *A* Platform, *B* retractable platform

delivered to the paws when subject completed the circuit by stepping to the shelf. After being shocked, subject was removed from the apparatus within 5 sec and etherized in a 50 ml glass beaker containing a gauze sponge saturated with diethyl ether. Subject was confined in the beaker, by means of a lucite cover, for approximately 36 sec. The anesthetized subject was then returned to its home cage to recover. Recovery time was approximately 25 sec.

Group II (Shocked-No Ether). Subjects were placed on the platform, and the same procedure as in Group I was followed, except that after stepping to the shelf and receiving the shock, subjects were immediately returned to their home cage.

Group III (Shocked-Ether, 1-Hr.). Subjects were placed on the platform, and the same procedure as in Groups I and II was followed, except that after stepping to the shelf and receiving the shock, subjects

were immediately returned to their home cage. One hour following the response, they were etherized in a glass beaker with diethyl ether. The anesthetized subject was then allowed to recover in its home cage.

Group IV (Unshocked-Ether). Subjects were allowed to step from the platform to the shelf, but received no shock. Within 5 sec. after stepping down subjects were etherized and returned to their home cage.

Group V (Unshocked-No Ether). Subjects were allowed to step from the platform to the shelf, receiving no shock for doing so. Immediately after stepping down, subjects were returned to their home cages.

Group VI (Unshocked-Ether, 1-Hr.). Subjects were allowed to step from the platform to the shelf with no shock. Subjects were immediately returned to their home cage. One hour following the response, they were etherized in the manner described above and allowed to recover in their home cage.

Response latencies were recorded for all subjects, beginning with the withdrawal of the shelf, and terminating with the descent of subject from the platform to the shelf.

24 hours following the training trial all subjects were tested on the apparatus. Groups I, II and III were tested for the retention of the conditioned response-avoidance of the shelf by not stepping from the platform, and Groups IV, V, and VI were tested as controls. The circuit was interrupted between platform and shelf so that no shock was delivered. Subjects were again placed on the platform; the timer was started when the shelf was pulled out, and stopped when subject stepped to the shelf. Subjects not stepping off the platform by 20 sec. were removed by hand.

Table. χ^2 *analysis for frequency of conditioned response in all groups*

Comparison	χ^2
Groups (all)	33.44[1]
Shock with ether vs. Shock no ether	12.73[2]
No shock with ether vs. No shock no ether	0.00
Shock with ether (1-Hr.) vs. Shock with ether	20.58[1]
No shock with ether (1-Hr.) vs. No shock with ether	0.13

Note: Except for 4 df between all groups there was 1 df for all other comparisons.

[1] $p < .01$.

[2] $p < .05$.

Results

The results indicate that ether anesthesia given immediately after the shock was effective in interfering with the acquisition of an avoidance response reinforced with a single punishing shock. Response latencies for all six groups, because of the 20 second cut-off point imposed, were analyzed by a χ^2 design. A conditioned response was considered to be one which showed a latency in excess of 10 sec. The analysis for the frequency of conditioned responding is shown in the Table.

The data presented in Fig. 2 illustrate the significant differences obtained. Significant differences were obtained between the anesthetized and unanesthetized shocked groups, and between the shocked groups immediately anesthetized and those anesthetized one hour following the conditioning trial. In the absence of shock, anesthesia had no effect on the response.

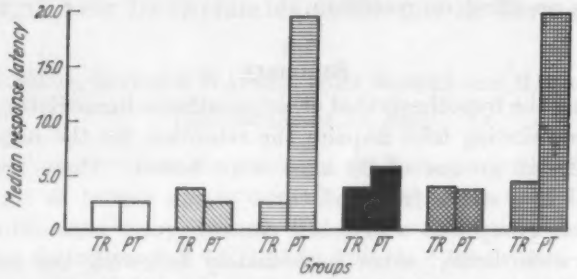

Fig. 2. Median response latencies for all groups on training and posttreatment trials. *TR* Training trial; *PT* Post-treatment trial. □ unshocked, no ether; ▨ unshocked, ether; ▤ shocked, no ether; ■ shocked, ether; ▩ unshocked, ether (1 hour); ▦ shocked, ether (1 hour)

Discussion

The results demonstrate the usefulness of the present technique for establishing a stable conditioned avoidance response in mice. The data suggest that anesthesia immediately following a single conditioning trial appreciably impairs the retention of the response upon testing 24 hours later. The fact that there is no difference between the unshocked groups indicates that an unconditioned response is unaffected by ether.

If as much as one hour intervenes between shock and anesthesia, the avoidance response is retained, indicating that the interval is sufficient for consolidation.

Whereas response latencies for subjects in the immediately etherized-shocked group were significantly reduced, there is still an indication that they tended to be elevated beyond those of the control group. This indicates that while there was a significant impairment of retention for the conditioned response, a minimal amount of responding did occur.

These data confirm previous findings (Pearlman et al. 1959) that immediately induced anesthesia causes retrograde amnesia. They cast doubt upon a statement by Burns (1958, p. 90) that rapidly induced anesthesia does not produce retrograde amnesia. The present findings contradict this finding and suggest that retrograde amnesia may be produced by a brief anesthesia immediately following a single conditioning trial. This finding is in accord with the view that the consolidation process, leading to the retention of recently acquired information, depends upon a minimal degree of neural activity.

One may conclude, on the basis of the data presented, that: (1) the single-trial conditioning technique described provides a convenient means of investigating the effects of several agents on retention in mice; (2) brief anesthesia with diethyl ether immediately following a response conditioned in a single trial impairs the retention of this response; (3) if the anesthesia is introduced one hour following the conditioning trial, it has no effect on retention.

Summary

In testing the hypothesis that ether anesthesia immediately following a single conditioning trial impairs the retention for the response thus conditioned, six groups of 20 mice were tested. Three groups were conditioned in a single trial and three groups served as controls. An experimental group and a matched control group were either: (1) anesthetized with diethyl ether immediately following the response (2) anesthetized one hour following the response or were (3) not anesthetized. The results showed that immediate ether anesthesia was effective in impairing the retention of the avoidance response. One hour post-conditioning etherization had no apparent effect on retention, and ether anesthesia did not alter the responses of control subjects.

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The Influence of Progesterone on Behavioral Changes Induced by Lysergic Acid Diethylamide (LSD-25) in Normal Males*

By

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(Received October 19, 1960)

This paper summarizes the results of the first of a series of studies on the influence of certain steroids in altering the effects of LSD-25[1] on psychological behavior in humans. The expectation that these steroids should alter the efficacy of LSD-25 in inducing behavioral changes in humans was derived from a number of animal studies (BERGEN et al. 1959; BERGEN and PINCUS 1960; BERGEN et al. 1960) which demonstrated first, that learned rope climbing in rats is temporarily impaired by LSD-25; and, second, that injection of certain steroids prior to LSD-25 administration inhibits the decrement in rope climbing ordinarily induced by LSD-25 alone. Of the variety of steroids studied, progesterone appeared to be one of the most potent in antagonizing this effect of LSD-25. Accordingly, it was chosen as the first steroid to be explored in our program concerning the efficacy of various agents in counteracting behavioral changes in humans demonstrated to be induced by LSD-25.

The general expectation in this study, then, was that progesterone would reduce the degree of behavioral change normally induced by LSD-25. While the counteractive effect of progesterone on LSD-25 was the primary focus of this investigation, the experimental design was such as to allow assessment of the possible psychological effects of progesterone alone as well as replicate some of our previous findings concerning the behavioral effects of LSD-25 (WAPNER and KRUS 1960b).

Method

Subjects. Twelve normal male adult volunteers of average and above average intelligence drawn from the personnel of Worcester Foundation for Experimental Biology participated in the study. Four Subjects were maintenance workers, four technical assistants and four research scien-

* This study was supported by PHS Grants MY 2262 and MY 2967 from National Institute of Mental Health, U.S. Public Health Service.

[1] LSD for this study, supplied by Sandoz Pharmaceuticals, Hannover, N J., was d-lysergic acid diethylamide tartrate in sealed ampules containing 0.1 mg. per ml.

tists. Their ages ranged from 26 to 38 years with a mean of 31 years. Subjects were screened to insure that none had a past history of emotional upset.

Experimental situations. The experimental situations utilized in this study were chosen on two grounds; first, that the behavior tapped in the situation change significantly under LSD-25; and second, that the degree of behavioral change be amenable to precise objective measurement. Experimental situations satisfying these criteria were chosen from among those previously utilized in the ongoing program of research at the Clark University laboratories concerned with the behavioral effects of LSD in normal and schizophrenic adults. Eleven situations — sampling sensori-motor, perceptual and conceptual behavior — were chosen to comprise the test battery employed in this study.

Sensori-motor Processes. 1. Steadiness Test: in which subjects must hold a stylus in a series of holes graded from large to small without touching the sides of the hole. The score taken is the number of times subjects touch the side of the hole for a 15-second trial in each of 5 holes. Under LSD it has been found that steadiness decreases (WAPNER and KRUS 1960b).

2. Two Hand Coordinator: in which subject must maintain contact between a movable button controlled by subject and a metal disc target traversing an irregular two dimensional pattern independent of subject's control. Subject controls the movable button by two lathe handles, one of which actuates left-right movement, and one which actuates front-back movement through the medium of rack and gear devices. The score taken reflects the time subjects maintain coincidence between button and target during the 60 seconds comprising a trial. Under LSD, two hand coordination as measured in this situation tends to decrease (WAPNER and KRUS 1960b).

3. Tapping Rate: in which subjects tap with a stylus at a speed comfortable to them. The score is the number of taps for a 15-second time interval. Under LSD, tapping rate increases (WAPNER and KRUS 1960b).

4. Card Sorting: in which subjects deal a standard deck of 52 playing cards into two piles at a speed which seems natural to them. The score taken is the duration of time required for the task. Under LSD, card sorting speed decreases (WAPNER and KRUS 1960b).

5. Handwriting: in which subjects are required to write a standard phrase (United States of America) at their normal handwriting speed. Score taken is the time to write the phrase. Under LSD, handwriting speed decreases (WAPNER and KRUS 1960b).

Perceptual Processes. 1. Apparent Horizon: in which subjects must adjust to apparent eye level in a dark room a black line horizontally bisecting a square patch of light. The score taken reflects the position

of the apparent horizon (apparent eye level) in relation to objective eye level. Under LSD, the influence of the position of the black line at the start of a trial is greater (final adjustment is closer to initial setting (WAPNER and KRUS 1960b).

2. Heiss-Sander Task: in which subjects must locate a designated part in a whole configuration made up of that part and other parts. The time taken by subject to extract the designated part from the whole is the score used. Under LSD, time taken to extract a part increases (KRUS and WAPNER 1959).

Conceptual Processes. 1. Simple Arithmetic: in which subjects must complete a series of simple addition problems (e.g., 1 + 2) at a speed which is comfortable to them. The score taken is the time subject requires for the task. Under LSD, speed of doing simple arithmetic decreases (WAPNER and KRUS 1960b).

2. Complex Arithmetic: same as above except that the series of problems is more difficult (e.g., 23 + 18). The score taken is the time subject requires for the task. Under LSD, speed of doing complex arithmetic decreases (WAPNER and KRUS 1960b).

3. Stroop Color-Word Test: in which subjects are presented with a card containing 100 color-name words (red, green, blue) printed in an ink whose color is incongruent with the printed name. Subject's task is to name the color of ink in which the color-name words are printed (e.g., the word "blue" printed in green ink to which subject must say "green" rather than "blue"). The score employed is the time taken by subject to name the 100 ink colors on the card. Under LSD, time taken to complete this task increases (greater interference) (WAPNER and KRUS 1960a).

4. Raven Progressive Matrices Test: in which subjects must abstract a general principle involved in a series of geometric designs in order to pick from a group of alternative designs the correct one to complete the series. The score taken reflects the time required to complete the test. Under LSD, time taken to complete the test increases (WAPNER and KRUS 1960b).

Procedure. The above battery of tests was administered to each of 12male subjects under each of four experimental conditions: placebo, progesterone, LSD, and progesterone preceding LSD. Since a double blind technique was employed, it was necessary that subjects be given what appeared to be identical agents on each testing day. This was accomplished by using the following regimen orally administered:

(A) Placebo Day: Placebo tablets containing 600 mg. sodium bicarbonate (four 150 mg. tablets) followed one hour later by 20 ml. of water.

(B) Progesterone + LSD Day: 600 mg. progesterone (four 150 mg. tablets) followed one hour later by 75 γ LSD in 20 ml. of water.

(C) Progesterone Day: 600 mg. progesterone (four 150 mg. tablets) followed one hour later by 20 ml. water.

(D) LSD Day: 600 mg. sodium bicarbonate tablets (four 150 mg. tablets) followed one hour later by 75 γ LSD in 20 ml. of water.

On each day, then subjects received 600 mg. (four 150 mg. tablets) of identically appearing pills followed one hour later by approximately 20 ml. of colorless, odorless, tasteless liquid. Sequence and order effects

Table 1. *Analyses of Variance*
Experimental situations

Source of variation	df	1. Steadiness		2. Two Hand Coord.		3. Tapping Rate		4. Card Sorting	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Bet. Ind's (l)	11	6846.9	< 1.0	122.8	2.06	1559.5	1.24	49.5	< 1.0
Sequence	3	1611.7	< 1.0	291.6	4.90[1]	2390.5	1.92	31.4	< 1.0
l/Seq. . .	8	8810.2	—	59.5	—	1247.9	—	56.2	—
Order . . .	3	1297.7	< 1.0	145.2	9.61[1]	16.4	< 1.0	2.6	< 1.0
Conditions .	3	5114.1	4.0[1]	48.4	3.22[1]	210.4	1.07	19.1	6.86[1]
Sq. Uniq. .	6	617.9	< 1.0	2.4	< 1.0	56.6	< 1.0	2.4	< 1.0
Residual . .	24	1276.8	—	15.1		196.4	—	2.8	—
Total . . .	47	2742.6	—	49.2		487.1	—	14.7	—

Source of variation	df	5. Handwriting		6. Horizon		7. Heiss-Sander		8. Simple Arith.	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Bet. Ind's (l)	11	595.4	1.01	94.1	< 1.0	17.8	1.08	585.1	1.07
Sequence	3	611.7	1.04	75.7	< 1.0	21.7	1.33	690.9	1.27
l/Seq. . .	8	589.2	—	101.0	—	16.4	—	545.4	—
Order . . .	3	12.6	< 1.0	108.3	4.73[1]	41.0	7.17[1]	215.9	7.55[1]
Conditions .	3	17.8	1.34	166.5	7.28[1]	10.2	1.79	168.7	5.90[1]
Sq. Uniq. .	6	5.6	< 1.0	70.6	3.09[1]	6.1	1.05	13.2	< 1.00
Residual . .	24	13.3	—	22.9	—	5.7	—	28.6	—
Total . .	47	148.8	—	60.3	—	11.1	—	177.8	—

Source of Variation	df	9. Complex Arith.		10. Stroop		11. Raven	
		Mean Square	F	Mean Square	F	Mean Square	F
Bet. Ind's (l)	11	3912.6	< 1.0	893.8	1.02	239108.6	1.05
Sequence	3	1975.9	< 1.0	947.6	1.08	272060.9	1.19
l/Seq. . .	8	4638.8	—	873.6	—	226751.5	—
Order . . .	3	546.4	8.87[1]	1431.8	18.64[1]	489932.1	6.12[1]
Conditions .	3	447.7	7.27[1]	564.1	7.34[1]	90182.9	1.13
Sq. Uniq. .	6	87.4	1.41	144.2	1.88	71705.0	< 1.0
Residual . .	24	61.6	—	76.8	—	80087.8	—
Total . . .	47	1021.8	—	394.2	—	143039.9	—

[1] $P < 0.05$.

of the experimental conditions were controlled by employing a 4×4 latin square design replicated 3 times, i.e., three subjects in each of the four sequences, ABCD, DABC, CDAB, and BCDA. One week was interpolated between each of the experimental conditions. Testing was begun two hours after the oral ingestion of the 20 ml. of liquid.

Results

Data were treated by computing a separate analysis of variance for each of the eleven experimental situations (Table 1). Results of these analyses are presented in summary form in Tables 2 and 3. Table 2 presents the mean scores and standard deviations for each of the dependent variables under each of the experimental conditions. Table 3 presents a summary of the analyses of variance performed on the data from each of the experiments, as well as an indication of whether the "progesterone" and "progesterone + LSD" conditions differed signifi-

Table 2. *Summary of Mean Scores and Standard Deviations*

Experimental Situation		Test Conditions			
		Placebo	Progesterone	Progesterone + LSD	LSD
1. Steadiness: No. of Hits (High Score indicates Unsteadiness)	M	22.2	32.2	38.1	70.0
	S.D.	25.5	47.8	44.4	67.5
2. Two Hand Coordinator: Time on Button Correctly (High Score indicates Greater Coordination)	M	49.8	47.8	46.5	45.1
	S.D.	4.4	6.9	8.8	5.9
3. Tapping Rate: Taps/15 secs (High Score indicates Faster Tempo)	M	71.2	69.3	76.8	78.4
	S.D.	19.0	23.3	24.3	19.0
4. Card Sorting: Time (secs) (High Score indicates Slow Speed)	M	23.7	24.9	24.2	26.6
	S.D.	3.6	3.6	3.3	3.9
5. Handwriting: Time (secs) (High Score indicates Slow Speed)	M	51.9	50.6	49.9	50.9
	S.D.	11.4	11.2	12.0	13.3
6. Horizon: Starting Position Effect in cms (High Score indicates Greater Effect)	M	9.5	12.5	11.8	18.2
	S.D.	7.3	5.4	7.7	7.4
7. Heiss-Sander Task: Time to locate part (secs) (High Score indicates Slow Speed)	M	8.1	8.0	9.8	9.6
	S.D.	2.2	2.1	5.2	2.3
8. Simple Arithmetic: Time (secs) to do problems (High Score indicates Slow Speed)	M	55.4	55.8	60.9	62.9
	S.D.	11.3	13.8	14.0	11.8
9. Complex Arithmetic: Time (secs) to do problems (High Score indicates Slow Speed)	M	81.2	81.0	89.1	92.6
	S.D.	30.6	33.8	29.9	30.2
10. Stroop Test: Time (secs) to read interference ~ rd (High Score indicates high interference)	M	89.1	92.4	100.8	105.1
	S.D.	13.8	17.8	22.3	19.9
11. Raven Test: Time to do test (secs) (High Score indicates Slow Speed)	M	449	540	573	659
	S.D.	102	291	345	569

Table 3. *Summary of Analyses of Variance*

Experimental Situation	Means in expected direction?	F ratio reflecting between conditions variance significant?	"*t*" test between "LSD" and "Progesterone + LSD" significant?
1. Steadiness	yes	yes	yes
2. Two Hand Coordinator	yes	yes	no
3. Tapping Rate	yes	no	no
4. Card Sorting	yes	yes	yes
5. Handwriting	no	no	no
6. Apparent Horizon .	yes	yes	yes
7. Heiss-Sander Task . .	no	no	no
8. Simple Arithmetic . .	yes	yes	no
9. Complex Arithmetic .	yes	yes	no
10. Stroop Test	yes	yes	no
11. Raven Test	yes	no	no

cantly from each other. This latter result is based upon a *t*-test of the significance of the differences between the means for each of these conditions based on the residual from the analysis of variance of the particular experimental situation.

The findings may be summarized as follows:

1. As can be seen from an examination of the means in Table 2 and the number of significant F ratios listed in Table 3, our previous findings concerning the direction of effect of LSD versus placebo was replicated in 10 of the 11 experiments, though "*t*" tests of the difference between placebo and LSD means were significant in only 7 of these.

2. With respect to the main problem of this study, i.e., whether progesterone would reduce the degree of behavioral change attributed to LSD, two findings are of pertinence: First, examination of Tables 2 and 3 indicates that in 9 of the 11 experiments the mean scores fall in the predicted direction, i.e., the mean score for the progesterone-LSD condition falls between the mean scores for the placebo and LSD conditions. Second, as can be seen from the summary of *t*-tests in the right hand column of Table 3, the differences between the mean scores for the LSD condition as compared to the progesterone + LSD condition are significant below the .05 level of confidence for 3 of these 9 situations.

3. While the question of whether progesterone itself has a behavioral effect is not the main concern of this study, comparison of behavior under progesterone and placebo is pertinent to this problem. Though none of the differences between the mean scores for the progesterone and placebo conditions are significant, it is interesting to note that there is a consistency to the direction of the differences. In 8 of the 11 experimental situations (all but situations 3, 7, and 9), the direction of dif-

ference between progesterone and placebo is the same as that between LSD and placebo. This trend is supported by some qualitative observations. Some subjects, for example, reported feelings of irritability, slowness and general depression on the days they had received progesterone. All of this suggests that it might be worthwhile to investigate more intensively whether progesterone alone induces behavioral changes.

Discussion

There is some evidence from this study, analogous to those of the animal studies mentioned earlier, to suggest that the magnitudes of behavioral changes found to occur following ingestion of LSD are reduced when LSD ingestion is preceded by ingestion of progesterone. Moreover, for the most part, this applies to behavior representing the sensorimotor and perceptual as well as conceptual levels of organization. Thus, the antagonistic effects of progesterone on LSD are not restricted to lower animals, or to a single area of behavioral functioning.

It should be noted that the nature of the relationship between progesterone and LSD is not delineated by these studies but will depend upon further research. Such research will have to consider, for example, whether progesterone merely slows in time the onset of maximal LSD effect, or whether it reduces the absolute magnitude of such effects. Since our studies employed a fixed sequence of experimental situations administered after approximately the same interval post drug ingestion, we have no evidence concerning this problem. Such evidence could be gathered in studies which would enable us to plot onset and recovery curves of the changes in behavior induced separately by progesterone and by LSD, as well as in interaction with each other.

Somewhat related to this problem is the question of whether or not progesterone in and of itself has an effect upon behavior. While progesterone is usually assumed to be inert insofar as psychological changes are concerned, there is a suggestion in the present study from the results presented above that this assumption might be questionable.

Answers to some of the preceding questions should enable us to more adequately conceptualize the psychological effects of progesterone as well as the nature of the interaction between progesterone and LSD. In previous publications, LSD has been viewed as a pharmacological means of inducing behavioral regression (Wapner and Krus 1960b), i.e., as a primitivizing agent which would be expected to lead to less mature behavior. From this viewpoint, the nature of the interaction between progesterone and LSD could be posed in terms of the question of whether or not there are agents like progesterone which arrest or perhaps reverse developmental regressions.

Summary

The influence of a steroid, progesterone, in altering the effects of LSD-25 on psychological behavior in normal male humans was investigated in this study. Some evidence was found that the magnitude of changes found to occur under LSD-25 in behavior representing sensori-motor, perceptual and conceptual levels of organization, was reduced when LSD ingestion was preceded by progesterone ingestion.

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Action of Phenothiazine Derivatives on Carbohydrate Uptake of Isolated Rat Diaphragm and Isolated Rat Spinal Cord

By

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(Received November 14, 1960)

Introduction

Chlorpromazine has been found by various workers to increase blood glucose levels in the experimental animal (NORMAN and HIESTAND, 1955; LINDAUR, 1956; RYALL, 1956) and in man (CHARATAN and BARTLETT, 1955; HILES, 1956). In insulin treated mice, chlorpromazine reduced the incidence of convulsions and death (NORMAN and HIESTAND, 1955; RYALL, 1956). In chlorpromazine treated rabbits, the blood sugar tolerance curve after glucose *per os* was flatter and more protracted than normally. On the other hand, the blood sugar curve after intravenous administration of glucose reached higher values than normally. LINDAUR explained these findings as a result of retardation of glucose transport, but from observations in intact organisms it is very difficult to ascertain how chlorpromazine exerts its influence on carbohydrate metabolism and we found it natural to investigate whether chlorpromazine had an effect on the glucose uptake of isolated tissues. Two isolated tissues were chosen, a muscle sample: rat diaphragm, and a sample belonging to the central nervous system: rat spinal cord. Isolated rat diaphragm has been used for innumerable studies of carbohydrate metabolism, and the spinal cord was chosen by us because it could be isolated relatively intact and with natural organ membranes over the greater part of its surface. The preservation of intact organ membranes was supposed to be of importance if it turned out that the effect of chlorpromazine on glucose metabolism was directly antagonistic to the effect of insulin, as it was found that insulin *in vitro* increases the glucose uptake of isolated rat spinal cord and some other samples belonging to the central nervous system, samples which are all covered with natural organ membranes on a large part of the surface (RAFAELSEN, 1958; 1961a—c).

Experimental

Animals. Female, non-pregnant, albino rats (Wistar strain) weighing 80 to 100 g. and fasted for 24 hours were used.

Anaesthesia. Anaesthesia was in most experiments produced by inhalation of a mixture of 50% carbon dioxide and 50% oxygen for

two to three minutes. In a few experiments the animals were anaesthetized by an intraperitoneal injection of Nembutal.

Procedure. Two tissue preparations were used: one of muscle and the other belonging to the central nervous system. The incubation procedure was identical, but in one type of experiment isolated rat hemidiaphragms, in the other isolated spinal cord pieces were incubated. The tissue preparations were carefully isolated exactly as previously described (RAFAELSEN, 1958, 1959). After isolation the tissue samples were treated in exactly the same way, whether they were hemidiaphragms or spinal cord pieces. The samples were soaked in ice-cold medium immediately after preparation. When all hemidiaphragms or spinal cord pieces were prepared they were transferred to incubation flasks; volume approximately 35 ml., and containing 2 ml. of medium. Each tissue sample was incubated separately. Aeration took place at 4° for 30 seconds with a mixture of 95% oxygen and 5% carbon dioxide. The flasks were shaken for 90 minutes at 37° in an incubation apparatus with an oscillation rate of 84—88/minute. The bicarbonate buffered medium was as described by GEY and GEY (1936), except that magnesium was omitted. It contained per 1.: 7.000 g. NaCl; 0.370 g. KCl; 2.270 g. $NaHCO_3$; 0.335 g. $CaCl_2$, 6 H_2O; 0.150 g. Na_2HPO_4; and 0.030 g. KH_2PO_4. The final concentrations of the individual ions were: Na^+ 148 mM, K^+ 5.2 mM, Ca^{++} 1.5 mM, Cl^- 128 mM, HCO_3^- 27 mM, and PO_4^{---} 1.3 mM. The glucose concentration in the medium was 300 mg./100 ml. In a few experiments glucose was replaced by D-fructose, D-galactose, or D-arabinose, all in a concentration of 300 mg./100 ml.

In each experiment 5 to 12 control hemidiaphragms or spinal cord pieces were incubated simultaneously with 5 to 12 hemidiaphragms or spinal cord pieces under the influence of one of the drugs investigated. Mean values for carbohydrate uptake were calculated for control hemidiaphragms or spinal cord pieces and hemidiaphragms or spinal cord pieces under the influence of drug.

Two main types of experiment were performed:

In one type, hemidiaphragms or spinal cord pieces from untreated rats were incubated in medium with or in medium without drug. "Pairing" of hemidiaphragms was performed so that from each animal one hemidiaphragm was incubated in medium with drug and the other hemidiaphragm from the same animal in medium without drug. In the spinal cord experiments, pieces of similar size were "paired" for incubation in flasks without drug and in flasks with drug, but no attempt was made to pair spinal cord pieces belonging to the same animal. The drugs employed were chlorpromazine, perphenazine, prochlorperazine maleate, prochlorperazine methansulfonate, imipramine, promazine, trimeprazine, methotrimeprazine, reserpine, meprobamate, ben-

Table 1. *Glucose uptake of hemidiaphragms from untreated rats incubated without and with chlorpromazine, perphenazine, prochlorperazine maleate, prochlorperazine methansulfonate, imipramine, promazine, trimeprazine, methotrimeprazine, reserpine, meprobamate, benactyzine, morphine, disulfiram, aprobarbital, pentobarbital, mescaline, LSD, and serotonin added to the medium*

Exp. No.	Name and concentration of drug in medium mg./100 ml.	No. of hemi-diaphragms	Glucose uptake Mean ± S.E.M.		Difference		Signif-icance of difference
			Without drug mg. of glucose/g. of wet diaphragm/hr.	With drug mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet dia-phragm /hr.	per cent	P
64	chlorpromazine 0.125	6 vs. 6	7.9 ± 0.10	7.7 ± 0.22	−0.2	− 3	>0.05
60	chlorpromazine 0.25	5 vs. 5	8.0 ± 0.60	7.0 ± 0.13	−1.0	−13	>0.05
64	chlorpromazine 0.25	6 vs. 6	7.9 ± 0.10	6.4 ± 0.25	−1.5	−19	*<0.001*
64	chlorpromazine 0.50	6 vs. 6	7.9 ± 0.10	6.9 ± 0.16	−1.0	−13	*<0.001*
60	chlorpromazine 1.0	5 vs. 5	8.0 ± 0.60	5.9 ± 0.55	−2.1	−26	*<0.05*
66	chlorpromazine 1.0	6 vs. 6	7.7 ± 0.31	5.8 ± 0.32	−2.1	−27	*<0.01*
106	chlorpromazine 2.0	12 vs. 12	4.9 ± 0.18	3.8 ± 0.23	−1.1	−22	*<0.01*
60	chlorpromazine 2.5	5 vs. 5	8.0 ± 0.60	5.8 ± 0.53	−2.2	−28	*<0.05*
55	chlorpromazine 2.5	6 vs. 6	6.2 ± 0.22	5.0 ± 0.27	−1.2	−19	*<0.01*
55	chlorpromazine 25	6 vs. 6	6.2 ± 0.22	4.8 ± 0.30	−1.4	−23	*<0.01*
54	chlorpromazine 25	6 vs. 6	5.6 ± 0.24	4.4 ± 0.16	−1.2	−21	*<0.001*
102	perphenazine 0.05	8 vs. 8	4.4 ± 0.31	3.9 ± 0.30	−0.5	−11	>0.05
102	perphenazine 0.10	8 vs. 8	4.4 ± 0.31	4.6 ± 0.31	+0.2	+ 5	>0.05
103	perphenazine 0.15	8 vs. 8	5.9 ± 0.29	4.3 ± 0.34	−1.6	−27	*<0.01*
101	perphenazine 0.20	8 vs. 8	6.7 ± 0.22	3.8 ± 0.23	−2.9	−43	*<0.001*
103	perphenazine 0.30	8 vs. 8	5.9 ± 0.29	4.0 ± 0.35	−1.9	−32	*<0.001*
101	perphenazine 2.0	8 vs. 8	6.7 ± 0.22	3.6 ± 0.36	−3.1	−46	*<0.001*
116	prochlorperazine maleate 2.5	12 vs. 12	7.6 ± 0.23	6.2 ± 0.29	−1.4	−18	*<0.001*
125	prochlorperazine maleate 2.5	10 vs. 10	5.6 ± 0.43	4.7 ± 0.50	−0.9	−16	*<0.05*
222	prochlorperazine methansulfonate 2.5	12 vs. 12	6.6 ± 0.15	6.0 ± 0.15	−0.6	− 9	*<0.01*
228	prochlorperazine methansulfonate 2.5	12 vs. 12	8.5 ± 0.23	7.8 ± 0.23	−0.7	− 8	*<0.05*
270	imipramine 5.0	11 vs. 11	5.9 ± 0.22	4.8 ± 0.18	−1.1	−19	*<0.01*
275	imipramine 5.0	11 vs. 11	4.9 ± 0.23	3.9 ± 0.19	−1.0	−20	*<0.01*
153	promazine 10.0	12 vs. 12	4.4 ± 0.17	4.2 ± 0.08	−0.2	− 5	>0.05
155	promazine 25.0	12 vs. 12	4.4 ± 0.16	4.4 ± 0.18	0	0	—
221	trimeprazine 2.5	12 vs. 12	6.4 ± 0.15	6.0 ± 0.19	−0.4	− 6	>0.05
226	methotrimeprazine 2.5	12 vs. 12	6.8 ± 0.30	6.6 ± 0.30	−0.2	− 3	>0.05
66	reserpine 0.001	6 vs. 6	7.7 ± 0.31	7.6 ± 0.28	−0.1	− 1	>0.05
66	reserpine 0.01	6 vs. 6	7.7 ± 0.31	7.6 ± 0.27	−0.1	− 1	>0.05
108	meprobamate 5.0	8 vs. 8	5.7 ± 0.31	5.4 ± 0.40	−0.3	− 5	>0.05
111	meprobamate 5.0	8 vs. 8	6.2 ± 0.23	5.8 ± 0.30	−0.4	− 6	>0.05
108	benactyzine 1.0	8 vs. 8	5.7 ± 0.31	5.9 ± 0.36	+0.2	+ 4	>0.05
111	benactyzine 1.0	8 vs. 8	6.2 ± 0.23	6.3 ± 0.52	+0.1	+ 2	>0.05
115	morphine 1.0	8 vs. 8	5.6 ± 0.29	5.1 ± 0.28	−0.5	− 9	>0.05
116	morphine 1.0	12 vs. 12	4.9 ± 0.33	4.6 ± 0.38	−0.3	− 6	>0.05
115	disulfiram 2.0	8 vs. 8	5.6 ± 0.29	5.2 ± 0.40	−0.4	− 7	>0.05

Table 1 (Continued)

Exp. No.	Name and concentration of drug in medium mg./100 ml.	No. of hemi-diaphragms	Glucose uptake Mean ± S.E.M. Without drug mg. of glucose/g. of wet diaphragm/hr.	Glucose uptake Mean ± S.E.M. With drug mg. of glucose/g. of wet diaphragm/hr.	Difference mg. of glucose/g. of wet diaphragm /hr.	Difference per cent	Significance of difference P
104	aprobarbital 5.0	8 vs. 8	6.2 ± 0.27	6.8 ± 0.19	+0.6	+10	>0.05
104	pentobarbital 5.0	8 vs. 8	6.2 ± 0.27	6.1 ± 0.17	−0.1	− 2	>0.05
147	mescaline 20.0	12 vs. 12	4.1 ± 0.14	3.8 ± 0.15	−0.3	− 7	>0.05
149	D-lysergic acid diethylamide 2.0	12 vs. 12	3.8 ± 0.17	3.5 ± 0.21	−0.3	− 8	>0.05
151	serotonin 2.0	12 vs. 12	4.1 ± 0.20	4.2 ± 0.19	+0.1	+ 2	>0.05

actyzine, morphine, disulfiram, aprobarbital, pentobarbital, mescaline, D-lysergic acid diethylamide, serotonin, and insulin.

In the other type of experiments (made only with muscle tissue samples), hemidiaphragms from rats given an intraperitoneal injection of either drug (chlorpromazine, prochlorperazine maleate, or methotrimeprazine) or saline before sacrifice were incubated in medium without drug. The volume injected was always 1 ml.

Analytical methods

Carbohydrate uptake. Glucose, fructose, and arabinose were determined by the method of NELSON and SOMOGYI (SOMOGYI, 1952). Galactose was determined by the same method, but with a longer boiling period (HAFT, MIRSKY and PERISUTTI, 1953). After incubation two samples of 0.100 ml. were drawn from the incubation medium in each flask for carbohydrate determination. Samples were also drawn from control flasks aerated and incubated without tissue. Carbohydrate uptake was calculated from the difference between the carbohydrate concentration in the control flasks and that in the flasks containing tissue samples. The uptake was expressed as mg. of carbohydrate/g. of wet diaphragm or wet spinal cord/hr.

Statistical analysis. All values in the tables after ± are expressions of S.E.M. (standard error of the estimate of mean value). The significance of difference between means has been established by calculating *t*. *P*, the probability of difference being due to chance, was obtained from tables for *t* (FISHER and YATES, 1943).

Results

The effect of various drugs on the glucose uptake of rat hemidiaphragms was studied in two ways. Table 1 shows the results obtained in experiments where the drugs were added to the medium in which

paired hemidiaphragms from untreated rats were incubated. It is seen that of the drugs investigated, only some of the phenothiazine derivatives were able to influence the glucose uptake. Chlorpromazine, perphenazine, and prochlorperazine are all derivatives of chlorphenothiazine, and these three drugs were all able to reduce the glucose uptake significantly. The reduction caused by chlorpromazine was of the order of 25% within a concentration range of 0.25 to 25 mg. chlorpromazine per 100 ml. in the incubation medium. Within this range there was apparently no correlation between chlorpromazine concentration and decrease of glucose uptake. The percentage decrease of glucose uptake seemed greater with prochlorperazine maleate than with the methansulfonate salt, but this problem was not investigated further. Except with chlorpromazine and perphenazine no attempt was made to study the minimal effective dose of the drugs. Some phenothiazine derivatives which do not contain chlorine in the aromatic nucleus: promazine, trimeprazine, and methotrimeprazine were unable to reduce the glucose uptake to a statistically significant degree. Imipramine, an iminodibenzyl derivative, did reduce the glucose uptake, but to obtain this effect a rather high concentration in the medium was necessary. A number of drugs outside the phenothiazine family were tried: reserpine, meprobamate, benactyzine, morphine, disulfiram, aprobarbital, pentobarbital, mescaline, D-lysergic acid diethylamide, and serotonin. None of these drugs influenced glucose uptake of isolated rat diaphragm.

Table 2. Glucose uptake of hemidiaphragms from control rats treated with saline and rats treated with chlorpromazine, prochlorperazine maleate, and methotrimeprazine

Exp. No.	Drug injected	Time from injection to sacrifice	Blood sugar at sacrifice in per cent of control group	No. of hemi-diaphragms	Glucose uptake, Mean ± S.E.M.		Difference		Significance of difference
					Saline treated animals	Drug treated animals			
	mg./100 g. body weight	hours	per cent		mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm/hr.	per cent	P
54	chlorpromazine 1.25	1½	118	6 vs. 8	5.6 ± 0.24	4.4 ± 0.17	−1.2	−21	*~0.001*
53	chlorpromazine 12.5	3	217	10 vs. 10	8.7 ± 0.47	7.0 ± 0.39	−1.7	−20	*< 0.02*
126	prochlorperazine maleate 6.25	12	179	12 vs. 12	5.0 ± 0.42	3.7 ± 0.30	−1.3	−26	*< 0.02*
119	methotrimeprazine 20.0	2½	134	12 vs. 12	3.7 ± 0.33	4.0 ± 0.41	+0.3	+ 8	> 0.05

Table 3. *Uptake of various sugars by hemidiaphragms from untreated rats incubated without and with chlorpromazine added to the medium. Chlorpromazine concentration in medium: 2.5 mg./100 ml.*

Exp. No.	Sugar	No. of hemi-diaphragms	Sugar uptake Mean ± S.E.M.		Difference		Significance of difference
			Without drug mg. of glucose/g. of wet diaphragm/hr.	With drug mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm per hr.	per cent	*P*
76	D-fructose	6 vs. 6	4.4 ± 0.23	3.5 ± 0.24	−0.9	−20	~*0.02*
76	D-galactose	6 vs. 6	3.6 ± 0.12	2.1 ± 0.28	−1.5	−42	<*0.001*
75	D-arabinose	8 vs. 8	3.5 ± 0.31	3.3 ± 0.15	−0.2	− 6	>0.05

In the second type of experiment the drugs were injected intraperitoneally to the rats before sacrifice, and glucose uptake of hemidiaphragms from these animals was compared with glucose uptake of hemidiaphragms from control animals injected with saline. In these experiments no drug was added to the medium. These experiments were performed only with chlorpromazine, prochlorperazine maleate, and methotrimeprazine (Table 2). Large doses of chlorpromazine were used: 1.25 and 12.5 mg./100 g. body weight. It is seen that mean blood glucose values were higher in drug treated animals than in control animals. The glucose uptake of hemidiaphragms from drug treated animals was smaller than that of the controls. The decrease was statistically significant.

The above mentioned experiments were all performed with glucose in the incubation medium. In experiments with other sugars than glucose in the incubation medium, it was found that the uptake of D-fructose and D-galactose in isolated rat diaphragm was reduced when chlorpromazine was added to the medium, whereas the uptake of D-arabinose was unchanged (Table 3).

In some experiments both chlorpromazine and insulin were added to the medium. Table 4 shows that insulin in sufficient doses is able to obviate the effect of chlorpromazine, but that even high insulin concentrations cannot exert the same effect when chlorpromazine is present as they do in the absence of this drug.

We have found (RAFAELSEN, 1958, 1961a) that insulin increases the glucose uptake of isolated rat spinal cord pieces. It was of interest to investigate whether chlorpromazine and other phenothiazine derivatives exerted an action on the glucose uptake of this preparation from the central nervous system. It can be seen from Table 5 that chlorpromazine, perphenazine, and prochlorperazine were able to reduce the glucose uptake in this preparation also. The minimal effective dose

of chlorpromazine was about 10 times as high with this preparation as in experiments with isolated rat diaphragm. In addition, Table 5 shows an experiment with D-galactose instead of D-glucose in the incubation medium, and it is seen that chlorpromazine also reduced the uptake of this sugar in isolated rat spinal cord pieces. Promazine was unable to influence the glucose uptake of isolated rat spinal cord as were mescaline, D-lysergic acid diethylamide and serotonin; a complete parallel to their lack of effect on isolated rat diaphragm. Imipramine, which exerted an effect on the glucose uptake of rat diaphragm, did not influence the glucose uptake of isolated rat spinal cord in the concentration used.

In this table is also shown a single experiment where the glucose uptake of brain slices was measured. In experiments to be reported elsewhere (RAFAELSEN, 1961b) it has been found that insulin increases the glucose uptake of "first" brain slices (= surface slices) to a moderate degree, whereas insulin has no effect on the glucose uptake of "second" brain slices. In the experiment presented here, first brain slices were used, and it is seen that addition of chlorpromazine to the incubation medium caused a decrease in glucose uptake. The decrease was small, but statistically significant.

Table 4. Glucose uptake of isolated rat diaphragm and isolated rat spinal cord incubated without and with insulin or chlorpromazine added to the medium. Chlorpromazine concentration in medium: 2.5 mg./100 ml. Number of hemidiaphragms or spinal cord pieces given in parenthesis after glucose values

Exp. No.	Preparation	Conc. of insulin in medium	Glucose uptake, Mean ± S.E.M.				Difference				Significance of difference
			− insulin − chlorpromazine	− insulin + chlorpromazine	+ insulin − chlorpromazine	+ insulin + chlorpromazine	D—B		D—C		
			A	B	C	D	mg. of glucose/g. of wet diaphragm per hr.	per cent	mg. of glucose/g. of wet diaphragm per hr.	per cent	*P*
		i.u./ml.	mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm/hr.	mg. of glucose/g. of wet diaphragm/hr.					
62	rat diaphragm	10^{-4}	7.4 ± 0.47 (6)	6.2 ± 0.50 (6)		6.2 ± 0.38 (6)	0	0			—
		10^{-2}				8.4 ± 0.38 (6)	+2.2	+36			*< 0.01*
59	rat diaphragm	10^{-4}			5.4 ± 0.25 (5)	4.4 ± 0.17 (5)			−1.0	−19	*< 0.01*
		10^{-2}			8.3 ± 0.43 (5)	5.8 ± 0.45 (5)			−2.5	−30	*< 0.01*
170	rat spinal cord	10^{-4}	5.5 ± 0.22 (10)	4.4 ± 0.20 (10)	5.4 ± 0.30 (10)	5.3 ± 0.23 (10)	+0.9	+21			*< 0.01*
173		10^{-2}	4.8 ± 0.15 (10)	3.9 ± 0.21 (10)	6.2 ± 0.28 (10)	5.0 ± 0.17 (10)	+1.1	+28			*< 0.001*

Table 5. *Glucose uptake of spinal cord tissue from untreated rats incubated without and with chlorpromazine, perphenazine, prochlorperazine maleate, imipramine, promazine, mescaline, D-lysergic acid diethylamide, and serotonin added to the medium. In one experiment* with spinal cord tissue the medium contained D-galactose instead of D-glucose. In one experiment** first brain slices were incubated in glucose containing medium instead of rat spinal cord tissue.*

Exp. No.	Name and concentration of drug in medium mg./100 ml.	No. of spinal cord pieces	Glucose uptake Mean ± S.E.M. Without drug mg. of glucose/g. of wet spinal cord/hr.	Glucose uptake Mean ± S.E.M. With drug mg. of glucose/g. of wet spinal cord/hr.	Difference mg. of glucose/g. of wet spinal cord/hr.	Difference per cent	Significance of difference *P*
79	chlorpromazine 1.0	8 vs. 8	2.0 ± 0.29	2.4 ± 0.36	+0.4	+20	>0.05
80	chlorpromazine 2.5	8 vs. 8	2.3 ± 0.11	1.7 ± 0.23	−0.6	−26	*<0.05*
81	chlorpromazine 2.5	8 vs. 8	3.3 ± 0.10	2.1 ± 0.25	−1.2	−36	*<0.001*
170	chlorpromazine 2.5	10 vs. 10	5.5 ± 0.22	4.4 ± 0.20	−1.1	−20	*<0.01*
173	chlorpromazine 2.5	10 vs. 10	4.8 ± 0.15	3.9 ± 0.21	−0.9	−19	*<0.01*
81	chlorpromazine 5.0	8 vs. 8	3.3 ± 0.10	1.5 ± 0.20	−1.8	−54	*<0.001*
188	chlorpromazine* 5.0	12 vs. 12	1.8 ± 0.19	0.9 ± 0.18	−0.9	−50	*<0.01*
174	chlorpromazine** 2.5	12 vs. 12	10.0 ± 0.36	8.8 ± 0.39	−1.2	−12	*<0.05*
123	perphenazine 2.0	12 vs. 12	3.7 ± 0.12	2.7 ± 0.12	−1.0	−27	*<0.001*
124	prochlorperazine maleate 2.5	12 vs. 12	2.8 ± 0.19	2.2 ± 0.23	−0.6	−21	*~0.05*
271	imipramine 5.0	11 vs. 11	4.0 ± 0.20	3.6 ± 0.17	−0.4	−10	>0.05
154	promazine 10.0	12 vs. 12	3.9 ± 0.10	4.1 ± 0.09	+0.2	+ 5	>0.05
156	promazine 25.0	12 vs. 12	3.8 ± 0.12	3.9 ± 0.12	+0.1	+ 3	>0.05
148	mescaline 20.0	12 vs. 12	3.5 ± 0.11	3.2 ± 0.13	−0.3	− 9	>0.05
150	D-lysergic acid diethylamide 2.0	12 vs. 12	2.5 ± 0.16	2.7 ± 0.19	+0.2	+ 8	>0.05
152	serotonin 2.0	12 vs. 12	2.2 ± 0.08	2.2 ± 0.06	0	0	—

Discussion

In our experiments, chlorpromazine and some other phenothiazine derivatives reduced the glucose uptake of isolated rat diaphragm and isolated rat spinal cord. It is wellknown that insulin *in vitro* increases the glucose uptake of isolated rat diaphragm (GEMMILL, 1940, and many subsequent workers), and we have found that this hormone also increases the glucose uptake of isolated rat spinal cord (RAFAELSEN, 1958, 1961a). Much evidence has accumulated in recent years in favour of the hypothesis that insulin acts directly on muscle by increasing the transport of glucose and some other sugars into the muscle cells (LEVINE, GOLDSTEIN, HUDDLESTUN and KLEIN, 1950; PARK et al. 1955, 1957, 1959). Results with samples from the central nervous system indicate that insulin has a direct action on this system and that insulin also acts on this system by increasing the transport of glucose and some other sugars into the brain and spinal cord (RAFAELSEN, 1961a and b; WOODS, HUNTER and BURK, 1958). The antagonistic effects of insulin and chlorpromazine on the glucose uptake of isolated diaphragm and

spinal cord may indicate that these compounds compete to influence the insulin sensitive system which transports glucose and some other sugars across the membranes and into the cells. This hypothesis is supported by the finding that chlorpromazine reduced the uptake of D-galactose and D-fructose as well as the uptake of D-glucose, whereas it was without an effect on the uptake of D-arabinose, that is to say that chlorpromazine is able to influence the uptake of some of the insulin-responsive sugars and that it shares with insulin the lack of influence on the uptake of D-arabinose.

It is difficult to see at the present time whether these observations of reduced glucose uptake by organized samples from muscle and central nervous system under the influence of chlorpromazine can be brought in relation to observations on a cellular or subcellular level. We shall only mention a few findings: Chlorpromazine inhibited cytochrome oxidase and ATP-ase in the experiments of BERNSON, NAMAJUSKA and BOSHES (1956), and chlorpromazine has been found to depress phospholipid turn-over in brain tissue *in vivo* (ANSELL and DOHMEN, 1956).

Blood chlorpromazine concentrations of patients treated with this drug have been calculated to be about 1 mg./100 ml., and in the present experiments similar or somewhat lower concentrations of chlorpromazine *in vitro* readily reduced the glucose uptake of the isolated tissue samples. GROSSI, PAOLETTI and PAOLETTI (1960) found that chlorpromazine in comparable doses increased the incorporation of acetate-1-^{14}C, mevalonic acid-2-^{14}C and glucose-U-^{14}C in brain phospholipid fatty acids. This effect was also evident after the *in vivo* administration of small doses of chlorpromazine (3.5 mg./kg. i.p.). MCILWAIN and GREENGARD (1957) found that respiratory and glycolytic response of brain slices to electrical pulses was inhibited by comparable levels of chlorpromazine and mepazine (the lastmentioned drug does not contain chlorine in the nucleus), whereas in the experiments cited in the previous paragraph it has usually been necessary to apply chlorpromazine in higher concentrations to obtain the various effects. The physiological implications of these differences in minimal effective doses are not obvious.

Over a 100-fold increase in the concentration of chlorpromazine in the medium the reduction of glucose uptake by rat diaphragm remained the same. A parallel observation was made in a study of the increase of glucose uptake by rat diaphragm in the presence of carbutamide (RAFAELSEN, 1959). In both cases this may mean that these drugs can only exert a limited effect on glucose transport, this effect being maximal with moderate doses. It might have been possible to obtain a dose response relationship by a scrutiny of the effect of the drugs in a small concentration range near the lower effective limit.

The three chlorphenothiazine derivatives investigated all exerted an influence on the glucose uptake of the isolated tissue samples, whereas

the phenothiazine derivatives without a chlorine atom in the aromatic nucleus did not. This shows that small changes in the composition of these drugs may completely alter their ability to influence the carbohydrate metabolism. We have earlier reported that some oral antidiabetic drugs, *e.g.*, carbutamide, increase the glucose uptake of isolated rat diaphragm (RAFAELSEN, 1959; RAFAELSEN and LUNDBÆK, 1959). Our results suggested that carbutamide exerted its influence on the glucose uptake of muscle *via* the same transport system as does insulin. Thus it seems as if some different groups of compounds have a direct effect on this transport system and may compete to influence it. It has been found with antidiabetic drugs as with phenothiazine derivatives that moderate changes in the constitution of the compounds alter their effect on carbohydrate metabolism. The hypoglycemic effect of oral antidiabetic drugs is very dependent on the length of the side chains attached to the aromatic nucleus and alterations of the length of the side chains can give substances with no effect on the glucose metabolism — or substances with a hyperglycemic effect (LOUBATIÈRES, 1946, 1955).

The effects of chlorpromazine on glucose metabolism in man are consistent with the hypothesis that this compound antagonizes insulin. Chlorpromazine significantly delays the return of blood glucose values to normal level after the intravenous injection of glucose (CHARATAN and BARTLETT, 1955). HILES (1956) observed that some psychotic patients developed transient hyperglycemia and glycosuria during chlorpromazine treatment; the signs disappeared upon cessation of drug. Furthermore, some patients with previously controlled diabetes showed higher blood glucose levels and increased glycosuria while they received chlorpromazine. The condition of these patients reverted to previous status when chlorpromazine therapy was discontinued. These observations are in accordance with our own. We have observed glycosuria and moderate hyperglycemia in fasting, non-diabetic psychiatric patients during chlorpromazine therapy and some diabetic patients needed higher insulin doses while under treatment with chlorpromazine. The good results obtained in cases of functional hyperinsulinism treated with chlorpromazine may also be due to the direct antagonism between insulin and chlorpromazine (VALLANCE-OWEN, 1960).

The action of some chlorphenothiazine derivatives on carbohydrate metabolism reported in this paper is probably quite coincidental to the psychotropic action of the same compounds. As so little is known of the mode of action of psychotropic drugs at the present time it is noteworthy that these drugs are to a great extent used for the treatment of psychotic patients who twenty years ago were treated with another compound influencing the carbohydrate metabolism, *i.e.*, insulin. It will be of interest to see whether any future group of psychotropic drugs exerts an influence on carbohydrate metabolism.

Summary

1. Chlorpromazine *in vitro* reduces the glucose uptake of isolated rat diaphragm and isolated rat spinal cord by 25%. Minimum effective dose of chlorpromazine was 0.25 mg./100 ml. in rat diaphragm experiments and 2.5 mg./100 ml. in rat spinal cord experiments. Perphenazine and prochlorperazine were also effective, whereas a series of other drugs — phenothiazine derivatives and others — were without effect on the glucose uptake of the two tissue samples used. Imipramine in high concentrations reduced the glucose uptake of rat diaphragm, but not that of rat spinal cord.

2- Chlorpromazine also reduced the uptake of D-fructose and D-galactose, but not of D-arabinose, in the isolated rat diaphragm.

3. When both chlorpromazine and insulin were present in the medium in rat diaphragm and in rat spinal cord experiments, the glucose uptake was dependent on the insulin concentration. Insulin in sufficient doses obviated the effect of chlorpromazine, but even in high doses did not exert the same quantitative effect on the glucose uptake in the presence of chlorpromazine as in the absence of this drug.

4. The results obtained are consistent with the assumption that chlorpromazine and other chlorphenothiazine derivatives influence the insulin sensitive glucose transport system in cell membranes.

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The Effects of Reserpine on a Strongly Conditioned Emotional Response

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With 1 Figure in the Text

(Received October 1, 1960)

Since the inhibition of a response induced by a conditioned emotional response (CER) is presumably a function of anxiety or emotion associated with pain, this form of behavioral suppression provides one useful technique for the study of a single drug or the comparative actions of many drugs. Briefly, it consists of the suppression of a stable response by the effects of a previously neutral stimulus which has been paired with a noxious condition. For example, in rats, suppression of stable lever pressing for food is finally obtained during a 4-minute tone which has been repeatedly terminated with the application of a brief, strong electric shock. These operations provide a definite model for one form of anxiety and a base from which drug effects can be assayed.

In a series of recently published articles, Brady (1956a, b) presented important data concerning the effcts of reserpine on CER. In general, the findings were that reserpine reduced the overall lever-pressing rate considerably and essentially eliminated the CER evoked by a 3-minute stimulus. These results are of special significance since the CER method has been used for studying the actions of many drugs and for the development of a psychological technique for screening potent analgesics. Thus far only those drugs reported to have significant analgesic actions have produced marked suppression of the CER (Hill et al., to be published). In considering Brady's method, it should be noted that the CER was of moderate strength only; lever pressing during the stimulus period was suppressed to approximately 25 percent of the pre-stimulus rate. Thus, the question could be raised as to whether the effects of reserpine in this study were contingent upon the strength of the CER. Consequently, the present study was conducted to test the action of this drug on a CER of the strength previously used by the present writers which reduced lever pressing essentially to zero.

Apparatus and Procedure

The modified Skinner Box (Skinner 1938) used in these experiments has been described by Hill et al. (1957). The most noteworthy changes, made to facilitate the recording of behavior during drug sessions, con-

sisted of a relatively small inner conditioning chamber with curved corners, diagonal grid bars, a lower lever, and delivery of food only when the lever returned to the "up" position. The conditioned stimulus was a 425-cycle tone emitted from a square wave generator at an amplitude which was held constant for all animals (a potentiometer setting of 35 on a 100 unit scale). An 0.5 second, 60 cycle, 60 volt electrical shock was used as the unconditioned stimulus.

The 26 Wistar albino rats used were reduced to 70 per cent of ad libitum weight and were deprived of food for 23 hours before each experimental session. All were trained to press a lever for food reward. When stable rates were established a CER was superimposed on the lever pressing response. The conditioning procedure, a modification of that developed by ESTES and SKINNER (1941) has been given in detail by HILL et al. (1957). Briefly, the CER was established by presenting a 4-minute tone which was terminated simultaneously with the application of a 0.5-second electric shock through the gridded floor. For each trial lever pressing rates were automatically recorded for three consecutive 4-minute periods: pre-tone, tone, and post-schock periods. After a strong CER had been established (approximating 100 per cent suppression) two groups of 10 animals each were matched on the basis of individual pre-tone rate and degree of CER, and for group means of these measures. A group was then randomly selected for the reserpine treatment. The group means for pre-tone rate, tone rate, and post-shock rate on the "control" day were: no-drug group, 64.6, 0.6, 68.4; and experimental group, 63.3, 0.8, 58.3. The schedule of testing, which continued over 18 consecutive days as shown in Fig. 1, was the same for both groups; they were run concurrently, the only difference in procedure being the subcutaneous administration of reserpine to the experimental animals. The first test was run four hours after the initial injection of 0.4 mg/kg. Thereafter, and throughout the study, BRADY's procedure, except for dosages, was followed in administering reserpine after each test with maintaining doses being given on non-test days. The dosage schedule in mg/kg follows: 2—7 days, 0.2; 8—10 days, 0.3; 11—12 days, 0.4; 13—14 days, 0.5; 15—18 days, 0.6 mg/kg.

The "inflection ratio" (BRADY 1956a), was used as a measure of restoration of lever pressing during the stimulus period. This ratio is determined by the formula, $\frac{B-A}{A}$, where A is the rate for the pre-tone period and B is the rate for the tone period. Any decrease in the number of lever presses during the tone period will be reflected by a negative value with total cessation of responding being shown by a -1.0 value. In the present study, 5 was added to all "inflection ratios" to avoid negative values in applying an analysis of variance for repeated measurements.

Results

Through the first seven days little change occurred in the initial inflection ratio values (—0.98 and —0.97) for either group. From this time the control group showed a progressive increase in the pre-tone rate and an accompanying decrease in strength of the CER which terminated with a mean inflection ratio of —0.75, approximately that shown by BRADY for control days. The reserpine treated group maintained the high initial pre-tone rate through the 12th day, or until the dose was raised to 0.5 mg/kg. The pre-tone rate slowed on this dosage, and when 0.6 mg/kg was finally used the rate dropped to approximately 50 per cent of the

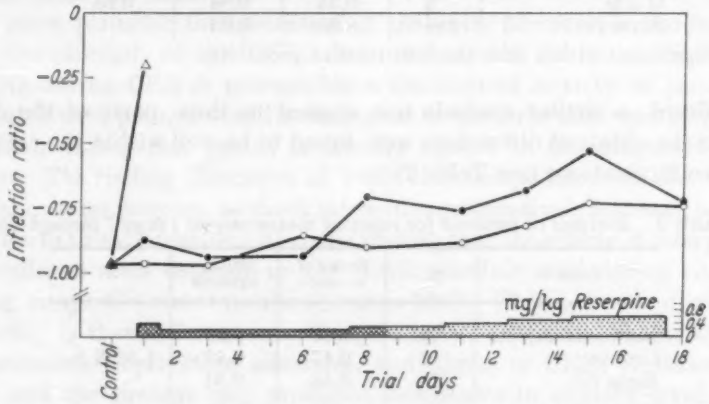

Fig. 1. Effects of reserpine and morphine on a strongly conditioned emotional response. ○ no-drug; ● reserpine; △ morphine

control value. This drop in pre-tone rate presumably reflects a generalized sedative effect of the drug on behavior. The mean inflection ratio, however, at the termination of this study remained a low —0.74. In BRADY's study the daily dose of reserpine was 0.2 mg/kg. It is important to note that the present study began with this dose, 0.2 mg/kg, but that the daily dose was periodically increased to a final level of 0.6 mg/kg, three times the dose level used by BRADY. Despite this increase, however, no significant effect on the CER was noticed. The non-significant trend for reserpine animals to exceed the no-drug animals in inflection ratios was contributed chiefly by three rats and at a time when CER strength was also decreasing in the no-drug group.

Table 1 summarizes the analysis of variance as applied to all inflection ratios of both groups. It shows clearly that the reserpine group did not differ from the no-drug group more than would be expected by chance. The table also shows that the change in the inflection ratios over time, although still moderate, was significant in both groups. The interaction between "groups" and "days" was not significant, however, showing

that the groups did not differ significantly on any test day. Since portions of the overall curve (days 8 through 18) might appear to be

Table 1. *Analysis of variance for repeated measurements (days 1 through 18)*

Source	df	Sums of squares	Mean squares	F
Between Subjects	19	4.61	0.24	1.00
Groups (G)	1	0.31	0.31	1.29
Error (b)	18	4.30	0.24	
Within Subjects	160			
Days (D)	8	2.26	0.28	4.66[1]
G × D	8	0.35	0.04	0.66
Error (w)	144	8.50	0.06	

[1] Significant at less than the 0.01 level of probablity.

significant, a similar analysis was applied to these parts of the data. Again the obtained differences were found to be well within the range of chance fluctuations (see Table 2).

Table 2. *Analysis of variance for repeated measurements (days 7 through 18)*

Source	df	Sums of squares	Mean squares	F
Between Subjects	19	6.05	0.32	
Groups	1	0.47	0.47	1.52 N.S.
Error (b)	18	5.58	0.31	
Within Subjects	80	6.54	8.18	
Days	4	0.45	0.11	1.38 N.S.
GxD	4	0.11	0.03	0.38 N.S.
Error (w)	72	5.98	0.08	

The inclusion of the change in the inflection ratio (—0.22) as a function of 7 mg/kg of morphine, obtained on a separate group, is given for comparative purposes only.

Discussion

Consistent with the reports of related studies (WEISKRANTZ 1956; STEIN 1956), the central findings of the present study, of course, is that chronic administration of reserpine did not significantly alter the inflection ratio of a CER from that found in a carefully matched control group of animals. However, differences in the findings of BRADY and the present authors might be a function of difference in strain of rats. This possibility could not be definitely "ruled out" since BRADY did not report the strain he used. Nevertheless, he reports in several other studies that Wistar strain, the same as employed here, was used; it is therefore assumed that differences in experimental outcome were not due to genetic differences. As indicated previously, the progressive rise in the inflection ratio shown in Fig. 1 presumably reflects some partial extinction of the

CER, since it occurred in both groups. Two interpretations of these results seem to warrant consideration.

One can be given in terms of qualitative differences of anxiety. It is becoming increasingly evident that a given drug may have differing effects on various types of escape, avoidance, and emotional responses. Thus it is possible that anxieties may be qualitatively different, depending upon the conditions which generate them. If this assumption is made it might be concluded that, despite the similarity in technique, BRADY's conditions produced a qualitatively different "anxiety" than that found in the present study and that reserpine has an ameliorating effect only on specific forms of anxiety.

A more plausible interpretation of the data, however, is concerned with the strength of the CER. Inasmuch as the inhibition of lever pressing during CER is presumably a function of anxiety or emotion associated with pain, it would seem logical to conclude that the extent of inhibition during this period is directly related to the magnitude of anxiety. The finding (BOREN et al. 1959) that escape latencies decrease, avoidance rates increase, as shock intensities progressively increase, lends credence to this contention. Also, the finding that the effects of reserpine on avoidance rates depends on the shock schedule used during conditioning, supports current results (SIDMAN 1956). Therefore, one obvious possibility is that differences in the parameters of electric shock, auditory stimulus, deprivation schedules, and degree of CER, of BRADY's study and the present one, produced differences in anxiety level. If this interpretation were accepted, it would seem that reserpine has little effect on strong levels of anxiety (present study), whereas on low levels of anxiety (BRADY's study) it has a significant effect. This alternative interpretation is preferred for several reasons: First, since both procedures appear quite similar, with the exception of the number of animals used, there appears to be little possibility of differences in "type" of anxiety between the two studies, although this possibility has not been definitely ruled out. Second, it is becoming more evident that the effects of drugs on behavior are not only sensitive to changes in the parameters involved but are also apparently quite specific in action.

For comparative purposes in connection with the specificity of drug actions, data on a potent analgesic from an unpublished study are shown (HILL, PESCOR and WIKLER). It can be seen in Fig. 1 that on the initial test day, four hours after injection, 0.4 mg/kg of reserpine had little or no effect on the CER, in comparison with 7 mg/kg of morphine 75 minutes after injection. The inflection ratio for the morphine group was —0.22, which represents a 78 per cent suppression of the CER, which of course was not approached by reserpine on any test day. The former is consistent with results on other opiates, and the latter, with the findings of this laboratory on non-analgesics.

Summary

Twenty-six Wistar albino rats were conditioned to inhibit a lever-presssig response during a 4-minute tone period which was terminated by a strong electric shock (CER). Two groups of 10 animals, matched on the basis of rate during the pre-tone and tone periods, were tested concurrently throughout the experiment. The experimental group was administered a daily and periodically increasing dose of reserpine (0.2—0.6 mg/kg) for a period of 18 consecutive days. It was found that reserpine produced no significant effects on the CER when compared with that of the matched control group, although an increase in the restoration of lever pressing as a function of time was found to be significant for both groups. It was concluded that the partial restoration of the lever-pressing response with time was a function of partial extinction of the CER, and that reserpine does not significantly reduce a strongly conditioned emotional response.

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Clinical Reports · Klinische Mitteilungen · Communications cliniques

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Chlorprothixene ("Truxal") Compared to Chlorpromazine

By

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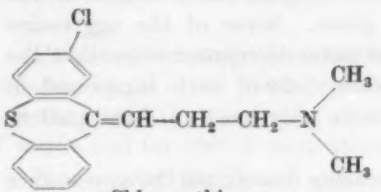
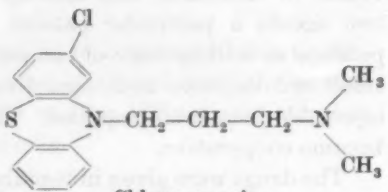
(Received November 16, 1960)

Recently reports have appeared concerning a new neuroleptic drug, *Chlorprothixene* (2-chloro-9-(3-dimethylaminopropylidene)-thiaxanthene, trans isomer), which seems to possess similar clinical qualities with the same or higher potency than chlorpromazine (MØLLER NIELSEN, PETERSEN and RAVN; MADSEN and RAVN), but with less pronounced side-actions (ARNOLD); the compound is registered as *Truxal* (N 714 trans, Lundbeck & Co. A/S, Copenhagen) and *Taractan* (Ro 4-0403, Hoffmann-La Roche & Co., Basel).



Chlorprothixene



Chlorpromazine

The present paper deals with the results of a comparative clinical study of the effect of chlorpromazine and chlorprothixene.

The Psychiatric University Clinic (Rigshospitalet) is nominated for short termed in-patient treatment, which means that the patients after two to three months' treatment have to be transferred either to out-patient treatment or to another psychiatric institution for further treatment.

To the clinic are admitted neurotic, acute as well as deteriorated chronic psychotic patients. Our standard treatment of psychotic states (except depression) and of major anxiety or aggression has for a time been chlorpromazine given in rapidly increasing doses until the appearance of clinical effect or disturbing side-effects, which often interfere with the treatment. For this reason our intention was to examine whether Chlorprothixene-treatment under the same conditions would be better in our routine treatment.

When two drugs, which one wants to compare, are effective against the same psychiatric conditions and probably have the same side-effects, it becomes possible, by preparing tablets or capsules, exactly alike, of the two drugs in equipotent qualities, to carry out "double-blind" test and register results as if only one type of treatment were being studied, and without changing the daily routine observation. Short-termed cross-over technique was not employed, because the beneficial effect as well as side-actions of treatment in one term might be carried over into the following term (SONNE).

Methods

The agents under trial were supplied to us by H. Lundbeck & Co. A/S in the form of small red capsules containing either 15 mg chlorprothixene ("Truxal") or 25 mg chlorpromazine and distributed in vials of 100 capsules. The vials were labeled with code numbers. Each patient treated in this trial was given his private vial. Irrespective of diagnosis and other personal data patients were alternately assigned to treatment with one or the other drug. The code was made in order to secure that each patient could be continued on the same drug during the entire course of treatment and nobody in the hospital knew which of the two agents a particular patient was given. Some of the aggressive patients as well as some of the paranoid patients refused to swallow the small red capsules and, therefore, coded vials of each compound in injectable form were supplied. These were only used until the patient became cooperative.

The drugs were given in rapidly increasing doses until the appearance of clinical effect or disturbing side-effects. Where clinical improvement or recovery appeared the dose was gradually reduced to a maintenance level, but in a few cases transference to other treatment was required, i.e. in schizophrenia: insulin coma, in manic states: lithium carbonate or ECT, and in depressions: ECT or imipramine.

If side-actions occurred, we tried to reduce the dose or changed the drug to a known agent — in most of the cases to chlorprothixene. During the treatment the effect and side-effects were evaluated and written on the ordinary records by the clinical staff and later discussed at staff meetings. At the end of the trial the results were tabulated before the code was opened.

Results and Discussion

The study comprises 179 patients of which 16 were excluded, because they refused to take the red capsules (this occurred before injectables were made available); four were previously treated with known chlorprothixene or chlorpromazine, one had ECT simultaneously, and one

erroneously was given injections of one of the agents and capsules of the other. The final group available for analyses consisted of *163 patients*, characterized with respect to age, sex and medication as seen in Table 2.

Table 1

	Paranoia		Mania		Hebephrenic and catatonic schizophrenia		Miscellaneous conditions[1]		Totally	
	t	c	t	c	t	c	t	c	t	c
Extremely rapid and pronounced improvement +++ . . .	1	1	2			1		1	3	3
Good improvement, symptomfree ++ .	11	6	3	2			1	4	15	12
Moderate improvement + ...	20	25	4	5	2	2	4	8	30	40
Unchanged 0	15	23	3	3	3	1	3	5	24	32
Aggravated	2			1				1	2	2
Totally	49	55	12	11	5	4	8	19	74	89

t = chlorprothixene (Truxal). c = chlorpromazine.

[1] Abstinence psychosis, delirium tremens, alcoholic hallucinations, emotional instability, hysteria with agitation and anxious depression.

Only in three cases the treatment had to be interrupted due to side-effects. In all other cases medication was continued until the patient left the clinic. The period of observation was for 127 patients less than 7 weeks and for only 3 more than 12 weeks.

Since neuroleptics have no causative effect against the mental diseases, but perhaps symptomatic effect against various mental symptoms and states, the results of treatment are arranged (in Table 1) according to mental states rather than to "diagnoses". Only the group listed under manic states and paranoia appear large enough to allow conclusion regarding the clinical effect.

Table 2. *Grouping of patients according to age, sex and medication*

Age (years)	Female		Male		Sum		Total
	t	c	t	c	t	c	
≧60	4	9	3		7	9	16
40—59	18	22	9	6	27	28	55
20—39	16	29	18	16	34	45	79
15—20	2	2	4	5	6	7	13
Sum	40	62	34	27	74	89	163

Of the 23 manics (12 chlorprothixene and 11 chlorpromazine) those on chlorprothixene showed somewhat better improvement than those on chlorpromazine. During the trial we often observed that the extremely manic patients first lost their happy mood and became angry and irritable and then subsequently they became quiet.

The 104 paranoid patients also showed somewhat better improvement during chlorprothixene treatment. With both agents it was found that the shorter the duration of the illness had been, the better improvement was obtained (Table 3). Differentiation in the degree of paranoia, of

Table 3. *Paranoid conditions, clinical effect in relation to duration of illness*

	> 2 years		1/2—2 years		2—6 months		< 2 months		Total	
	t	c	t	c	t	c	t	c	t	c
Extremely rapid and pronounced improvement +++ . . .	1	1							1	1
Good improvement symptomfree ++ .	1	2			5		5	4	11	6
Moderate improvement + ...	8	10	3	3	4	6	5	6	20	25
Unchanged 0	5	5	4	5	3	8	3	5	15	23
Aggravated			1		1				2	
Sum	15	18	8	8	13	14	13	15	49	55

hallucinations and of anxiety did not yield any additional pertinent information. The more severe the symptoms were, the more uncertain was the improvement, but during the treatment we often found that the very paranoid, hallucinated patient first of all became tranquillized. The emotional involvement with his delusions was reduced; however, delusion might still be present, but the patient was no longer troubled by its presence. Later the delusions became something which "had been". It was still a reality for the patient, but it was "a long time ago". Even some patients told that their delusions, which in their own opinion must have been a result of a psychotic disorder, were absolutely gone.

Side-effects (Table 4) are reported less frequently by the patients on chlorprothixene than on chlorpromazine medication. Particularly, the toxic side-actions and complications were rare.

Three of the chlorprothixene patients became a *little stiff in the face*, but showed no other extrapyramidal symptoms, whereas 14 of the chlorpromazine patients showed frank or extreme parkinsonoid symptoms.

Three chlorprothixene patients got slight *skin rashes* whereas 10 of the chlorpromazine patients got rashes in varying degrees, 7 of them very pronounced, which made it necessary to discontinue the treatment.

Two patients became *jaundiced*. The first after five days of chlorpromazine treatment, showing the common signs of liver function disorder. After stopping the treatment the jaundice and liver disorder

disappeared in three weeks, after which the patient was given chlorprothixene with good effect and no side-actions. The other case of jaundice appeared in a patient who had received chlorpromazine about three weeks and then, due to an administration error, was given chlor-

Table 4. *Side-effects (and daily dosis when it occurred)*
(Some of the patients showed more than one side-effect)

	Chlorprothixene				Chlorpromazine			
	60 to 120 mg	180 to 240 mg	300 to 360 mg	> 360 mg	100 bis 200 mg	300 to 400 mg	500 to 600 mg	> 600 mg
Jaundice	1(?)[1]				1(?)[1]	1		
Skin rashes		1 slight and before 3rd week	2		1	1	8	
Parkinsonoid symptoms			2 slight	1		6	8	
Orthostatic hypotension dizziness syncope	2	3	6		2	10	4	
Tachycardia (observed by the patient) dyspnoea					4			
Tiredness	2	5	9			11	4	1
Dryness of mouth							2	
Tremor		1				1		
Paresthesia		1				1		
Tinnitus						1		
Oedema and facial skin rash						1		
Feeling of derealization						1		
Fever without leucopenia		1						

[1] See text.

prothixene the last five days. The patient and her husband were so annoyed that she left the hospital the following day leaving no opportunity for liver function test. No information on the further course of the condition is available. The genesis of this case of jaundice is uncertain. If it is caused by the drugs, it may either be caused by five days treatment with chlorprothixene, or it may more probably be caused by the three weeks of chlorpromazine medication.

Table 5. *Maximal daily doses*

	Chlorprothixene				Chlorpromazine			
	60 to 120 mg	180 to 240 mg	300 to 360 mg	> 360 mg	100 to 200 mg	300 to 400 mg	500 to 600 mg	> 600 mg
Number of patients	4	34	26	10	6	32	36	15

In the *less serious side-effects* there seems to be *no difference* between the two drugs given in therapeutic doses.

There has been no case of agranulocytosis and no mortality.

It appeared that in treatment with daily doses higher than 200 mg chlorpromazine more than 50% showed side-effects, whereas not even the highest chlorprothixene dose provoked side-effects in 30% of the cases (Tables 4 and 5).

Summary

In a "double-blind" comparative study between chlorprothixene (Truxal) and chlorpromazine treatment in hospitalized, relatively acute patients we found that among all psychotic patients (except the depressive) as well as the hyperactive, physically agitated patients, the 74 chlorprothixene treated patients showed equally good or better improvement than the 89 chlorpromazine treated.

The minor side- effects appeared to be equally frequent during the two treatments, but chlorprothixene seemed to show no serious toxic effects, and particularly the extrapyramidal side-effects were so rare and so little pronounced that we found chlorprothixene to be preferable to chlorpromazine. It should be pointed out that the period of observation was relatively short (in 127 patients less than 7 weeks).

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Short Communications · Kurze Originalmitteilungen
Communications brèves

Notes sur l'inactivité neuroleptique d'un dérivé phénothiazique à propriété anti-apomorphine pure

Étude pharmacodynamique et clinique de la diméthylsulfamido-3 [(Méthylsulfonyl-4″ Piperazino)3′ Propyl]-10 Phénothiazine (9.260 R.P.)

Par

P.-A. Lambert, S. Courvoisier et L. Julou

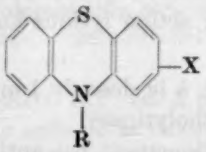
(Manuscrit reçu le 3 août 1960)

L'étude pharmacologique des amines dérivées de la phénothiazine comme la chlorpromazine et la prochlorpémazine a permis de mettre en évidence, parmi les nombreuses propriétés de ces substances, l'activité anti-apomorphine (Courvoisier et coll., 1953 et 1957, Ducrot et Koetschet, 1956).

Freedman et Giarman (1956) ont même suggéré que le test à l'apomorphine (Chen et Ensor, 1950) pourrait servir à la sélection d'un produit actif dans les psychoses. Or, parmi les très nombreux dérivés de la phénothiazine étudiés par nous, il en est un, le 9.260 R.P. ou diméthylsulfamido-3 [(méthylsulfonyl-4″ pipérazino) 3′ propyl]-10 phénothiazine qui ne montre pratiquement au laboratoire que la seule activité anti-apomorphine. Il était donc particulièrement intéressant, compte tenu de l'hypothèse émise par Freedman et Giarman, d'étudier ce dérivé en clinique, en vue de vérifier si l'activité anti-apomorphine élevée du 9.260 RP s'accompagnait ou non d'une action psychothérapeutique.

Étude pharmacologique (Courvoisier, Julou)

Tableau 1. *Structure chimique des 4.560 R.P., 6.140 R.P. et 9.260 R.P.*



N° R. P.	X	R
4.560 R.P. (chlorpromazine)	—Cl	$-(CH_2)_3-N(CH_3)_2$
6.140 R.P. (prochlorpémazine)	—Cl	$-(CH_2)_3-N\langle\rangle N-CH_3$
9.260 R.P.	$-SO_2N(CH_3)_2$	$-(CH_2)_3-N\langle\rangle N-SO_2-CH_3$

La toxicité aiguë du 9.260 R.P. sur la souris est beaucoup plus faible que celle de la chlorpromazine (4.560 R.P.) et de la prochlorpémazine (6.140 R.P.): voici les DL_{50} des trois produits par diverses voies, après trois jours d'observation:

Tableau 2. *Toxicité aiguë des 9.260 R.P., 6.140 R.P. et 4.560 R.P.*

Voie	DL_{50} mg/kg[1]		
	9.260 R. P.	6.140 R. P.	4.560 R. P.
i.v.	310 ± 15	92 ± 6	75 ± 5
i.p.	480 ± 80	125 ± 15	112 ± 12
s.c.	sup. à 2500	350 ± 110	300 ± 100
p.o.	1300 ± 200	750 ± 100	350 ± 50

[1] FINNEY, D. J.: Probit analysis. Cambridge: Cambridge University Press 1952.

L'étude de la toxicité chronique du 9.260 R.P., effectuée chez le rat et le chien, aux doses journalières de 20 et 40 mg/kg/p.o. pendant un mois, a permis de conclure à la très bonne tolérance générale du produit.

Le 9.260 R.P. paraît pratiquement sans action sur le système cardio-vasculaire: aux doses de 1 mg/kg/i.v. chez le chien chloralosé et de 5 mg/kg/i.v. chez le lapin uréthanisé, il n'exerce pas d'effet hypotenseur; dans les mêmes conditions, la prochlorpémazine et surtout la chlorpromazine sont plus hypotensives. Le 9.260 R.P. à la dose de 1 mg/kg/i.v. ne provoque pas d'altération du tracé électrocardiographique du chien chloralosé et n'engendre aucune tachycardie.

Le 9.260 R.P. se montre remarquablement bien toléré par le système orthosympathique du chien chloralosé, n'exerçant à la dose de 1 mg/kg/i.v. aucun effet vis-à-vis de l'hypertension consécutive à l'injection intraveineuse d'adrénaline ou de noradrénaline ou à l'occlusion bilatérale des carotides communes et n'engendrant pas d'hypotension orthostatique. Au contraire, à cette même dose, la prochlorpémazine et surtout la chlorpromazine manifestent une action lytique sur le système orthosympathique.

Chez le chien chloralosé, à la dose de 1 mg/kg i.v., le 9.260 R.P. est dénué d'action parasympatholytique.

Le 9.260 R.P. n'est pratiquement pas antihistaminique: à la dose de 20 mg/kg s.c., dans la technique de BOVET-STAUB, il ne protège pas les cobayes contre l'administration intraveineuse de 100 doses mortelles d'histamine.

Il n'est pas spasmolytique, dans la technique de MAGNUS sur l'intestion isolé du lapin, à la concentration de 10 mg/litre, à la fois sur le spasme à l'acétylcholine et sur le spasme au chlorure de baryum.

Il est dépourvu d'activité analgésique sur la souris, dans la technique de HESSE, aux doses de 40 mg/kg s.c. et 80 mg/kg p.o.

Tableau 3. *Action centrale des 9.260 R.P. prochlorpémazine (6.140 R.P.) et chlorpromazine (4.560 R.P.)*

Tests [1]	Animal	Doses [2] mg/kg ou mg/l	Voie	9.260 R. P.	6.140 R. P. prochlorpémazine	4.560 R. P. chlorpromazine
Potentialisation de la narcose à l'éther . .	souris	DE_{50}	s.c.	sup. à 250	20	4
	souris	DE_{50}	p.o.	110	—	14
Potentialisation de la narcose barbiturique	souris	DE_{50}	s.c.	sup. à 250	14	6
	souris	DE_{50}	p.o.	sup. à 250	—	8
Potentialisation d'analgésie à la morphine	souris	DE_{50}	s.c.	100	12	5
	souris	DE_{50}	p.o.	sup. à 500	—	10
Action hypothermique	souris	DE_{50}	s.c.	sup. à 500	8,5	5
	souris	DE_{50}	p.o.	450	—	12
Action antisérotonine in vitro	utérus de ratte	CE_{50}	in vitro	6,5	0,8	0,4
Test de la bataille . .	souris	DE_{50}	s.c.	19	—	7,5
	souris	DE_{50}	p.o.	23	8	4,0
Test de la traction . .	souris	DA_{50}	s.c.	sup. à 180	—	7
	souris	DA_{50}	p.o.	sup. à 80	25	13
Test de WINTER et FLATAKER	souris	DE_{50}	p.o.	80	5,5	4,0
Test du labyrinthe . .	rat	DE_{50}	p.o.	voisine de 120	16	19
Test du réflexe conditionné	rat	DE_{50}	p.o.	100	5,0	5,0
Action anti-apomorphine	chien	DE_{50}	s.c.	0,08	0,15	0,50
	chien	DE_{50}	p.o.	0,05	0,25	1,50
Action cataleptique. .	rat	DE_{50}	s.c.	750	15	50
	rat	DE_{50}	p.o.	500	—	100

[1] Pour le détail des techniques utilisées se reporter aux publications antérieures (COURVOISIER et coll., 1953 et 1957).

[2] Par convention, nous désignons par DA_{50} (dose 50% active) et par CA_{50} (concentration 50% active) la dose ou la concentration qui donne l'effet pharmacologique recherché chez 50% des animaux traités; nous appelons d'autre part DE_{50} (dose 50% efficace) ou CE_{50} (concentration 50% efficace) la dose ou la concentration qui donne un effet pharmacologique égal à 50% de l'effet pharmacologique maximum.

L'action centrale du 9.260 R.P. est à peu près inexistante et négligeable en comparaison de celle de la chlorpromazine et de la prochlorpémazine (tableau 3); ce produit ne possède pratiquement pas d'activité sédative, dépressive ou cataleptique. La seule propriété qu'il manifeste au niveau du système nerveux central est son activité anti-apomorphine: le 9.260 R.P. a une DE_{50} de 0,080 mg/kg/s.c. et 0,050 mg/kg/p.o. se montrant ainsi par voies sous cutanée et orale respectivement 2 et 5 fois plus actif que la prochlorpémazine; cette activité anti-apomorphine déjà très importante en elle-même apparaît encore plus remarquable si l'on considère l'absence quasi-totale d'activité du produit dans tous les autres tests d'action centrale.

Étude clinique (Lambert)

C'est dans le courant de l'année 1958 que, au sein du Comité Lyonnais de Recherches Thérapeutiques en Psychiatrie, nous nous sommes intéressés à l'étude du 9.260 R.P. Après quelques essais de tolérance qui montrent que le médicament est bien supporté, l'application en fut faite à des états psychotiques aigus, c'est-à-dire supposés facilement réversibles. Nous ne donnons ici que les observations les plus démonstratives.

Obs. 1: Can... Henri, 29 ans, bouffée délirante avec anxiété, hallucinations auditives, idées mystiques, tentatives de défénestration, mydriase, R.O.T. vifs, langue saburrale, insomnie. A reçu récemment de l'isoniazide pour une tuberculose pulmonaire qui semble actuellement stabilisée.

Du 10. 12. 58 au 17. 12. 58, il reçoit 150 mg I.M. de 9.260 R.P. Légère somnolence, une lipothymie, hypotension orthostatique, nausées et surtout fièvre vers 38—39°. Continue d'entendre des voix quoique de façon atténuée, reste anxieux. On donne à la suite: chlorpromazine 150 mg I.M., amélioration rapide, sort le 24 janvier 1959.

Obs. 2: And... Augustine, 54 ans, syndrome hypomaniaque, il s'agit de la 2° admission depuis le 23 avril 1956. Logorrhée, fuite, des idées, euphorie: «je travaille du casque, fou joyeux, mais pas furieux», subexitation.

Le 18. 11. 58, 250 mg p.o. de 9.260 R.P., phases de somnolence entrecoupées de phases d'agitation nécessitant le recours à une injection de diméthazine. Le 19. 11. 58, 300 mg de 9.260 R.P. Le 20. 11. 58, 450 mg. Même comportement nettement agité, on a recours à nouveau à une injection de diméthazine.

Arrêt du 9.260 R.P. le 21. 11. 58. On donne 7.843 R.P. (thiopropérazine) 30 mg I.M. La malade se met à dormir profondément, il faut la réveiller pour manger le 22. 11. et dans l'après-midi elle fait une crise excito-motrice avec protrusion de la langue. Evolution favorable de l'accès en trois jours sous l'effet du 7.843 R.P.

Obs. 3: Mic... Andrée, 38 ans, après admission pour état hypomaniaque, reste trois jours sans traitement, le comportement reste identique: improvisation de chansons, exubérance, volubilité, insomnie à noter des lésions psoriasiques discrètes. A déjà été traitée à deux reprises pour manie.

Le 5. 12. 58 reçoit 150 mg de 9.260 R.P. I.M. en trois fois, somnolence dans les deux heures qui suivent chaque injection, excitation ensuite. Maintien des 150 mg I.M. par jour jusqu'au 13. 12. 58. Passe par les mêmes périodes: somnolence et subexcitation selon le rythme des injections. Sécheresse de la bouche, malade assoiffée, douleurs aux points d'injection, température qui s'élève progressivement à 38° le 14. 12. 58. Amélioration de l'état mental. Le 14. 12. 58, arrêt de la voie I.M. On donne 300 mg p.o. Le 15. 12. 58, nausées, amaigrissement de 3 kg depuis le début, fièvre, état mental normal. Arrêt du traitement. Le 16. 12. 58 la T° est à 40°,1, l'état mental est toujours bon, mais la malade est abattue. Le 17. 12. 58, apparition d'un rasch scarlatiniforme. Le 18. 12. 58 chute de la température à 37°. Ensuite disparition progressive de l'éruption. Dans ce cas il y a tout lieu de supposer que l'amélioration mentale est la conséquence de la fièvre, c'est-à-dire de l'effet secondaire des injections de 9.260 R.P.

Obs. 4: Zim... Georgette, 47 ans, syndrome hallucinatoire chez une éthylique, «c'est une machination, mes pensées ne m'appartiennent plus, ma propriétaire les répète tout fort, d'autres me disent des mots malpropres». Cet état a été en s'accentuant depuis plusieurs mois. N'a jamais eu aucun traitement.

Le 22. 11. 58 on donne 9.260 R.P. 450 mg p.o. Le 26. 11. 58 on continue à lui parler, mais l'excitation psychique est moindre. Le 1. 12. 58, 9.260 R.P. 600 mg

p.o. Le 6. 12. 58 les hallucinations auditives restent nombreuses, mais la malade les supporte plus calmement. Pas de troubles neurologiques ou somatiques. Un peu de somnolence, se dit «abattue». Arrêt du 9.260 R.P., le 7. 12. 58, on donne à la place chlorpromazine 300 mg p.o. Le 15. 12. 58 les hallucinations ont totalement disparu.

Obs. 5: Bov... Emilie, 38 ans, rechute de syndrome schizophrénique. Déjà hospitalisée en 1955 et 1957. Forme catatonique avec mutisme (mais parle d'abondance sous eunoctal intraveineux), conserve la position assise plusieurs heures de suite.

Le 12. 2. 59, 9.260 R.P.: 100 mg I.M. et 200 mg p.o. Le 25. 2. 59, même comportement avec opposition aux soins. Arrêt du 9.260 R.P., on donne 7.843 R.P.: 30 mg I.M. Une semaine après est très améliorée.

Conclusions

Ces observations montrent que la seule activité du 9.260 R.P. sur le plan psychique est de provoquer une certaine sédation, d'ailleurs de courte durée le plus souvent. Ainsi, le 9.260 R.P. dont la seule activité centrale, sur le plan laboratoire, est l'activité anti-apomorphine importante, n'a manifesté aucune action dans les psychoses. Il apparaît alors que, dans la série des amines dérivées de la phénothiazine, il n'y a pas parallélisme entre l'activité anti-apomorphine et l'activité dans les syndromes psychotiques; cette conclusion rejoint celle de Pierre et Cahn (1957), à savoir que «l'action anti-apomorphine ne suffit pas discriminer une activité neuroleptique».

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The Excretion of Noradrenaline and Adrenaline in the Urine of Rats during Chronic Morphine Administration and during Abstinence

By

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With 1 Figure in the Text

(Received October 1, 1960)

In an earlier report (GUNNE 1959) dealing with the level of catecholamines in the rat brain during morphine abstinence, the possibility was advanced that the abstinence syndrome may have some relation to the hypothalamic level of catecholamines. Certain symptoms of central excitement, produced by chemical interference with the metabolism of monoamines of the brain, e.g. the effects of reserpine after administration of a monoamine oxidase inhibitor, have many features in common with the morphine abstinence syndrome. The noradrenaline level of the rat brain was shown to decrease ($P<0{,}05$) during the first few days of abstinence after three weeks of morphine treatment, and to return to normal within a week.

MAYNERT and KLINGMAN (1960) have observed a decrease of the catecholamine level in the brains of morphine tolerant dogs and rabbits after inducing an abstinence syndrome by nalorphine. In the rat brain, however, they did not find any decrease of the catecholamines.

The abstinence syndrome appearing in morphine addicts on withdrawal from large doses of the drug, is characterized by a markedly disturbed function of the autonomic nervous system. Indicative of this are symptoms like tachycardia, perspiration, tremor, mydriasis, piloerection, fever and mental excitement.

For the rat the reports have been rather contradictory. Some authors deny the existence of abstinence irritability in this species (SOLLMAN 1924, MYERS 1931, KAYMAKCALAN and WOODS 1956), while others have found symptoms with many similarities to those of man. JOËL and ETTINGER (1926) reported observable signs of abstinence excitement and the irritability of the rat was recorded objectively by BARLOW (1932), who measured the struggling of rats tied down in an uncomfortable position. With a modification of this technique HIMMELSBACH et al. (1935) recorded an abstinence irritability in the rat which roughly corresponded in duration to the abstinence syndrome of man. It lasted for

10 to 11 days. HANNA (1960) pointed out the significance of frequent morphine injections in order to establish a rapid tolerance and to obtain a noticeable abstinence syndrome in the rat.

The aim of the present study has been to examine the activity of the sympathetic system by following the urinary excretion of noradrenaline and adrenaline in the rat during a cycle of morphine administration for three weeks, as well as after withdrawal. Special attention has also been devoted to the behavioral changes in the various phases.

Methods

20 female albino rats, weighing 140—160 g at the beginning of the experiment, were divided into four groups of 5 animals. Three groups were later receiving morphine, while one was kept as a control and injected with saline throughout the experiment. Each group was kept in a cage constructed for the collection of urine, with arrangements for separation of fecal solids. The 24-hour urine was collected in bottles containing, as a preservative, enough 1 N sulfuric acid to keep p_H below 4. The animals were given intraperitoneal injections twice daily at 9 a.m. and at 5 p.m.

Before the morphine injections were started all rats received physiological saline until, after about two weeks, the daily urine level of catecholamines was reasonably constant.

Morphine hydrochloride was then given for 22 days in doses increasing stepwise every 7th day. During the first week the dose was 15 mg/kg, the second week 45 mg/kg and during the third week 135 mg/kg twice daily. After a final dose of 300 mg/kg given twice on the 22nd day of morphine administration (in order to preclude with reasonable certainty any admixture of abstinence phenomena at this stage) the injections of physiological saline were resumed for about two weeks.

The 24-hour urine of each group of rats was collected and analysed daily for noradrenaline and adrenaline content according to the method of EULER and LISHAJKO (1959). Recovery experiments yielded 62—82 per cent of added amounts of noradrenaline and adrenaline, the mean of 7 experiments with noradrenaline being 71 per cent. In the values presented below no correction has been made for losses of catechols in the procedure. When morphine hydrochloride was added to two urine samples, in amounts corresponding to the doses employed, it did not influence the determination of the catecholamines.

Results

Preinjection irritability was noticed in the rats during the morphine treatment when the same dose had been maintained for 5—6 days. This sign disappeared again when the dose was increased. During the ab-

stinence period, when the animals were given saline injections, this irritability was very marked and the rats were sometimes even aggressive.

The urinary catecholamine level of the saline treated controls remained fairly constant during the experiment and corresponded well to the levels of the other groups before the morphine administration. The mean values (± S.E.) for these saline injected rats were: noradrenaline 84 ± 2,9 ng/kg/hr and adrenaline 17,4 ± 0,9 ng/kg/hr (72 samples analysed).

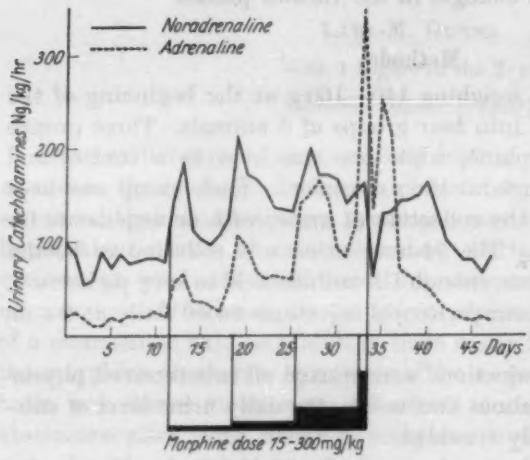

Fig. 1. Daily record of urinary catecholamines of rats before, during and after morphine administration. Each point in the curve represents the mean of 3 groups of rats, each comprising the pooled urines of 5 animals. The morphine dose was increased stepwise at weekly intervals: 15, 45, 135 and finally 300 mg/kg. When morphine was not given the animals received injections of physiological saline

Fig. 1 illustrates the effect of morphine. Both for noradrenaline and adrenaline there was a marked increase on the first few days at each new morphine dose, followed by a drop when the same dose had been repeated for some days. After five days of morphine both amines had almost returned to the normal level. Then there was a second smaller increase on the last day of this dose[1]. At about this time the rats had begun to show their irritability before the morning injection. When the dose was raised there was another sharp increase of adrenaline and noradrenaline, followed by another drop and the same pattern reappeared at the third dose level.

After a final dose of 300 mg/kg the morphine injections were replaced by physiological saline for two weeks. During the first abstinence day the catecholamine values were remarkably reduced as compared with the large excretion on the last morphine day, and the noradrenaline level even became subnormal. After this reduction there was another increase of both amines. Adrenaline, increasing as much as 15 times the normal, reached its peak on the second and third abstinence days, while for nor-

[1] Accidentally the samples of the sixth day on the 45 mg/kg dose level were destroyed and had to be discarded. The difference between the lowest excretion (5th or 6th day of each dose) and the excretion of the 7th day was for the 15 and 135mg/kg doses: noradrenaline 35 ± 13,1 ng/kg/hr and adrenaline 32 ± 9,6 (means ± S.E.). $P < 0{,}05$

Table 1. *Adrenaline excreted in the urine of rats during abstinence after 22 days of morphine administration. Significance level for difference between means and 72 control values: 17,4 ± 0,9 ng/kg/hr (mean ± S.E.)*

Abstinence day No.	Adrenaline ng/kg/hr Group No. I	II	III	Mean ± S.E.	Significance level
1	85	188	130	134 ± 30	***
2	366	202	185	251 ± 58	***
3	209	234	160	210 ± 22	***
4	65	79	63	69 ± 5,0	***
5	66	64	60	63 ± 1,8	***
6	66	56	51	58 ± 4,4	***
7	44	52	57	51 ± 3,8	***
8	44	34	42	40 ± 3,1	***
9	28	20	40	29 ± 5,8	*
10	23	20	22	22 ± 0,9	***
11	19	16	17	17 ± 0,9	N.S.
12	14	15	15	15 ± 0,3	*
13	17	16	20	18 ± 1,2	N.S.
14	16	10	18	15 ± 2,4	N.S.

Table 2. *Noradrenaline excreted in the urine of rats during abstinence after 22 days of morphine administration. Significance level for difference between means and 72 control values: 84 ± 2,9 ng/kg/hr (mean ± S.E.)*

Abstinence day No.	Noradrenaline ng/kg/hr Group No. I	II	III	Mean ± S.E.	Significance level
1	50	78	64	64 ± 8,1	*
2	66	152	168	129 ± 32	N.S.
3	107	166	124	132 ± 18	*
4	173	123	133	143 ± 15	***
5	180	130	123	144 ± 18	**
6	190	137	120	149 ± 21	**
7	135	181	170	162 ± 14	***
8	142	133	125	133 ± 4,9	***
9	90	86	129	102 ± 14	N.S.
10	98	77	95	90 ± 6,6	N.S.
11	88	78	74	80 ± 4,2	N.S.
12	86	80	70	79 ± 4,7	N.S.
13	69	69	60	66 ± 3,0	***
14	86	83	75	81 ± 3,3	N.S.

* $P < 0{,}05$; ** $P < 0{,}01$; *** $P < 0{,}001$; N.S. Not significant.

adrenaline the rise was slower, the highest level, about twice normal, being reached on the seventh day. Tables 1 and 2 give the values of the individual cages, means ± S.E. and the significance levels for the differences between these means and the controls.

Discussion

A single injection of morphine results in a decrease of the catecholamines in adrenals (Elliott 1912, Stewart and Rogoff 1922, Outschoorn 1952) and brain (Vogt 1954, Gunne 1959, Maynert and Klingman 1960) of cats and rats. Vogt obtained a similar effect with ether, nicotine, picrotoxin, insulin and β-tetrahydronaphtylamine. The action of these substances on the catecholamines is of short duration and in certain respects different from that of reserpine, which seems to act essentially by impairing the storage of amines. The morphine doses employed in these studies were generally rather high and the results may be regarded as a response of autonomic parts of the nervous system to the severe stress of acute intoxication.

The present study reveals an increased autonomic activity, as evident by a rise in the urinary excretion of adrenaline and noradrenaline during the first few days of regular administration of morphine. This occurs even in doses that do not affect the tissue levels of catecholamines. As

a result of tolerance the initial catecholamine excretion is diminished when the same morphine dose is repeated for some days. WARTBURG and AEBI (1960) observed a corresponding decrease in the catecholamine response of rats given chronic alcohol doses. — Whenever the morphine dose is raised, there is a corresponding increase of the catecholamines. The very marked adrenaline excretion on the highest morphine dose, 300 mg/kg, indicates a greatly increased adrenomedullary activity at this stage.

In addition to the stress of intoxication, a second factor seems to be involved in the long-term experiment, resulting in an increase of the catecholamine excretion whenever the morphine satiation of the animals becomes insufficient. Thus, after about five days of morphine administration, the rats have an increasing preinjection irritability and at the same time there is a slight increase of the urinary excretion of both catecholamines. These signs of physical dependence can be observed at the end of the injection periods with the same morphine dose.

After withdrawal of morphine the abstinence phase was accompanied by a markedly increased excretion of catecholamines. At this stage characteristic symptoms occurred consisting of piloerection, tremor, diarrhoea. The animals were lying inactive during the daytime, with their eyes closed. They were remarkably irritable on handling, shrieking when lifted in the tail and sometimes trying to bite even without being touched. In the evenings and during the night they were often fighting.

The symptoms reach their peak on the second and third abstinence days, simultaneously with the highest urinary excretion of adrenaline. Since the increased urinary level is generally accompanied by a decreased tissue level of noradrenaline in the brain (GUNNE 1959), it seems reasonable to assume a central and peripheral release, possibly combined with an impairment of the resynthesis or storage of catecholamines in the abstinence phase.

The brain level of 5-hydroxytryptamine and the urinary level of 5-hydroxyindole acetic acid do not indicate a release of 5-hydroxytryptamine during morphine abstinence in the rat (to be publ.).

In attempts to elucidate the effect of catecholamines on behaviour, the interest has been focussed on various substances which act on the brain level of the amines without clouding consciousness to a considerable degree. Signs of excitement and autonomic disturbance have been reported after administration of reserpine (effecting a release of stored amines) as well as after the catecholamine precursor dihydroxyphenylalanine (DOPA) in animals pretreated with monoamine-oxidase inhibiting substances, causing a delayed break-down of the amines (CARLSSON et al. 1957, VOGT 1959).

There is now considerable evidence supporting the view that a stage of excitement and vegetative disturbance can be elicited by a central release of catecholamines especially in combination with an impaired break-down.

The complex effects of acute morphine administration can only to a limited degree be ascribed to the concomitant release of catecholamines. In the abstinence phase on the other hand, the released amines act on a nervous system unprotected by any drugs and this time the symptom picture is entirely different. To what extent the abstinence syndrome in this and other species can be ascribed to a release of catecholamines, remains to be settled. The release observed here may prove to be a secondary biproduct of some other as yet undetected phenomenon.

Summary

The urinary excretion of noradrenaline and adrenaline was increased during the first few days of morphine administration in rats.

Following the initial rise, the urinary excretion of both amines decreased during one week of treatment with the same dose (tolerance phenomenon); however, on increasing the morphine dose, the cyclic pattern of initial rise and subsequent fall in catecholamine excretion reappeared.

During abstinence after three weeks of morphine administration, the animals where markedly irritable and presented characteristic vegetative symptoms. At the same time there was an increased excretion of adrenaline, amounting to 15 times normal, and a twofold rise of the noradrenaline excretion, both lasting for about 10 days.

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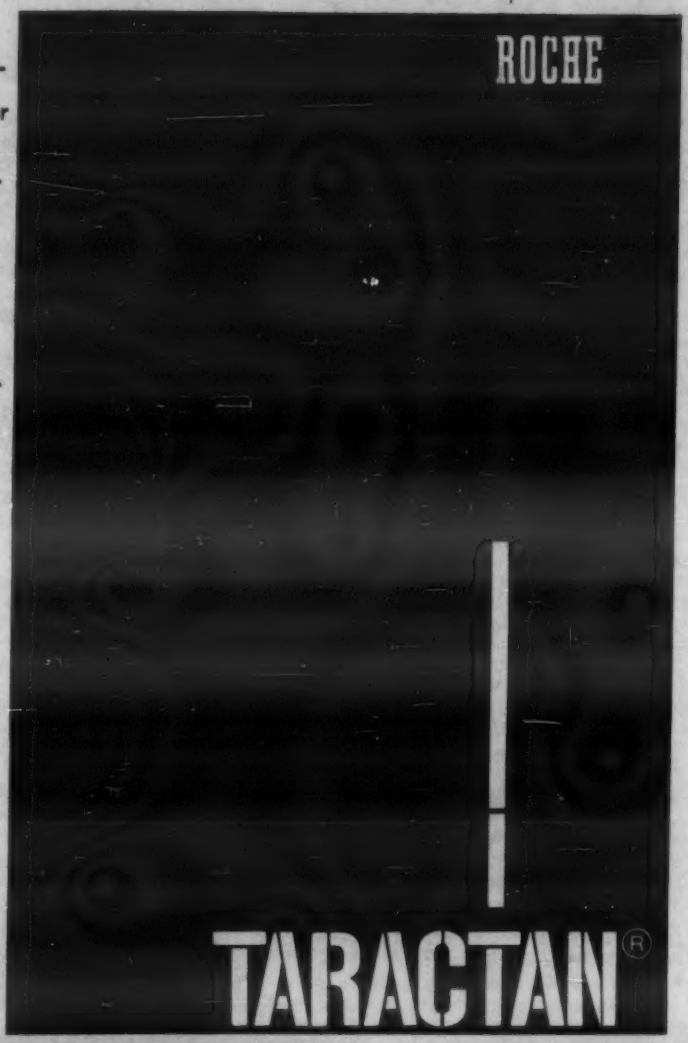

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